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ERRATA

Page 204. The figures in table 4 should be multiplied by 2 to convert them from parts per million of nitrates in the solution, to parts per million in the dry soil. This correction should be applied also to the figures in the paragraph next to the last on page 205.

Page 413. Title of paper should read: "The Colorimetric Determination of Soil *Nitrates* in a Colored Water Extract."

NITROGEN LOSSES UNDER INTENSIVE CROPPING[1]

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New Jersey Agricultural Experiment Station

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In an earlier paper attention has been called to the loss of nitrogen and carbon in cultivated land as compared with the loss on land abandoned to grass and weeds. The work covers a period of 9 years, and deals with a loam soil (inclining to a sandy or gravelly loam) which during the period of the experiment received only moderate applications of a nitrogenous fertilizer. A partial bibliography was included.

The work reported in this paper covers a period of 20 years and deals with a loam soil which, with certain exceptions to be noted later, has been heavily fertilized and manured regularly throughout the 20 years.

As originally planned the experiment included a study of the availability of nitrogen in different materials, as measured by the amount of nitrogen won back through the crop, the percentage of nitrogen in the crop as affected by the treatment, denitrification studies, and the effect of the various treatments on the income and outgo of nitrogen in the soil.

Fairly complete reports of this work have been published from time to time (4, 5), but these do not give the results of the nitrogen determinations in the soil later than 1912. It is proposed in this paper to consider particularly the nitrogen income and outgo of the soil.

The work has been carried out by means of cylinders which have already been described (4). The arrangement of these cylinders and the plan of fertilizer and manure treatment are shown in figure 1. The cylinders were installed and the first crop grown in 1898.

A 5-year rotation consisting of corn, oats (2 years), wheat and timothy has been carried out on the cylinders. Four such 5-year periods were completed with the timothy crop of 1917.

Two residual crops (a crop immediately following the main crop) have been grown during each 5-year period, but the fertilizers and manure are always applied for the first crop.

Cow manure was used at the rate of 16 tons per acre; nitrate of soda at the rate of 160 and 320 pounds per acre; dried blood and ammonium sulfate were used in amounts equivalent to 320 pounds of nitrate of soda per acre.

[1] Paper No. 23 of the Journal Series, New Jersey Agricultural Experiment Stations, Department of Soil Chemistry and Bacteriology.

[2] The authors are indebted to Dr. H. C. McLean, formerly Research Chemist, and Mr. A. L. Prince, Assistant Chemist, for a large part of the analytical work reported in this paper.

Series.

1. Check.
2. Minerals.
3. Minerals, manure, solid, fresh.
4. Minerals, manure, solid and liquid, fresh.
5. Minerals, manure, solid, leached.
6. Minerals, manure, solid and liquid, leached.
7. Minerals, nitrate of soda, 5 gms.
8. Minerals, nitrate of soda, 10 gms.
9. Minerals, manure, solid, fresh; nitrate, 5 gms.
10. Minerals, manure, solid, fresh; nitrate, 10 gms.
11. Minerals, manure, solid and liquid, fresh; nitrate, 5 gms.
12. Minerals, manure, solid and liquid, fresh; nitrate, 10 gms.
13. Minerals, manure, solid, leached; nitrate, 5 gms.
14. Minerals, manure, solid, leached; nitrate, 10 gms.
15. Minerals, manure, solid and liquid, leached; nitrate, 5 gms.
16. Minerals, manure, solid and liquid, leached; nitrate, 10 gms.
17. Minerals, sulphate of ammonia, equivalent to 10 gms. nitrate.
18. Minerals, dried blood, equivalent to 10 gms. nitrate.
19. Minerals, manure, solid, leached; sulphate of ammonia as in 17.
20. Minerals, manure, solid, leached; dried blood as in 18.

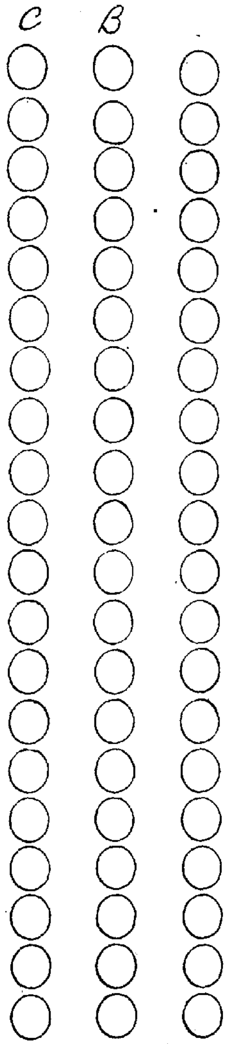

FIG. 1. DIAGRAM OF EXPERIMENT

All cylinders except the three of series 1 have received annual applications of acid phosphate at the rate of 640 pounds, and muriate of potash at the rate of 320 pounds per acre. Experience has shown that such applications furnish an excess of phosphoric acid and potash and thus make it unnecessary to take into consideration the amount of these constituents contained in the manure.

In certain cases the nitrogenous fertilizers were used alone and in others they were used in combination with the manure. The applications of manure are unusually heavy for general farm practice, but this was a part of the plan for denitrification studies.

When the soils were first prepared and placed in the cylinders a liberal application of lime was made to all, but no lime was again applied for 10 years. Thus for the first 10 years the treatment for the three cylinders (treatments in triplicate) of any given series was the same, and therefore, the results for each series of three cylinders could be averaged. In the spring of 1903—beginning of the second 5-year period—the soils of each series were removed and thoroughly mixed in one heap in order that slight differences might be equalized, and then one-third of this mixture returned to each of the three cylinders constituting a series. This procedure was repeated in the spring of 1908, that is, after 10 years of cropping. At this time there was placed in each cylinder the equivalent of 174.1 pounds of air-dry soil. Up to this time the treatment for the three cylinders of a series had been kept uniform. From this time forward a distinction is to be made. Those cylinders designated as *A* are to remain continuously unlimed; those designated as *B* and *C* are to be limed once every five years, and the *C*'s, in addition, are to grow two green-manure crops (legume) during the 5-year period.

These green-manure crops follow the corn crop and the first residual crop. In accordance with this plan the *B* and *C* cylinders received 125 gm. of pulverized limestone each, in the spring of 1908. Later this application was considered insufficient and the treatment was repeated in 1909. Aside from the lime and green manure, the three cylinders of a series receive like treatment.

Beginning in 1908 each season has given 60 distinct crops (one for each of the 60 cylinders), or a total of 600 crops covering the period 1908 to 1917, inclusive. To this number must be added 60 residual crops for each of the two 5-year periods, or a total of 720 crops for the 10 years.

A careful record has been kept of the amount of fertilizer and manure applied to each cylinder, and also of the dry matter in the form of crops removed from each. It is thus possible to calculate how much nitrogen has been applied during the 20 years and how much has been removed in the form of crops.

Nitrogen was determined in the original soil, and determinations have since been made on samples collected at intervals of about 5 years. It is thus possible to give a fairly complete inventory of the nitrogen income and outgo for the 20-year period.

As pointed out in an earlier paper (5) there has been a loss of about 40 to 70 per cent of the applied nitrogen as computed by taking the difference between the total amount applied and the amount won back through the crops.

Such losses may occur through the loss of nitrogen compounds in the drainage waters and also through the escape of gaseous nitrogen and ammonia into the air. Our knowledge of the proportions which are lost through one of these channels as compared with the other is limited. The problem of getting at the amount of loss through the drainage waters is not such a difficult one, and has already been studied by a number of investigators in this country and abroad, but a determination of the relative amount of nitrogen lost as nitrogen gas and as ammonia is a more difficult proposition.

In the work reported here no attempt has been made to measure *directly* the nitrogen that is lost through the channels mentioned, but the subject is considered from the standpoint of nitrogen gains or losses as indicated by the analysis of the soil and of the crops that are taken off, and the original nitrogen content of the soil plus the nitrogen applied in fertilizers and manure.

LOSS OF NITROGEN FOR THE FIRST TEN-YEAR PERIOD

The average results for the first 10 years (first and second rotations) are shown in table 1. This table shows the amount of nitrogen that was originally present in the soil, the amount that was applied to each cylinder during the 10 years, the amount that was removed by the crops and finally the total loss exclusive of the amount removed by crops.

The greatest loss was 39.38 gm. from series 12 and the least 25.12 gm. from series 19, with an average of 32.24 gm. It may be pointed out that the loss was almost as great from those cylinders that receive large applications as from those that receive small applications of nitrogen. It is generally true however, that there was less nitrogen removed in the crops from those cylinders that receive light applications than from those that receive heavy applications.

The average loss—32.24 gm. per cylinder—is equivalent to 1032 pounds per acre if the entire depth of the soil (about 10 inches) is included.

This means an average loss of 103 pounds of nitrogen per acre annually. This is an unusually heavy loss, but it must be remembered that the treatment to which the soils were subjected also was unusual to the extent that they were shoveled over more than would occur under field conditions and thus oxidation processes were accelerated.

It may also be pointed out that from soils rich in nitrogen, the loss is great. It is well known that there is a tendency toward equilibrium in soils. A poor soil left to itself may gradually increase its nitrogen content while a rich soil that is under constant tillage is very likely to decrease.

Discussing this tendency to equilibrium, Warington (9) says:

We have spoken earlier in this lecture of the natural limits to the accumulation of nitrogen in the soil: we can now, I think, perceive some of the causes of such limits.

The addition of organic matter to a soil either as crop or weed residue, or as farmyard manure, at once makes that soil a suitable home for the animal life, the fungi, and the bacteria whose function it is to reduce organic matter to the condition of inorganic matter. An increase of organic plant residue or manure thus creates some of the conditions favorable to its own destruction. The rate of oxidation in the soil is now no longer what it was; the oxidizing agents have increased with the material to be oxidized. If, therefore, a soil is laid

TABLE 1

Loss of nitrogen from cylinder soils during the first ten years—Average of sections A, B, and C

SERIES	*a* PRESENT 1898	*b* APPLIED 1898-1907	*m* $(a+b)$ TOTAL PRESENT AND APPLIED	*c* REMOVED 1898-1907	*d* PRESENT 1907	*n* $(c+d)$ TOTAL REMOVED AND PRESENT	$m-n$ LOSS EXCLUSIVE OF CROPS 1898-1907
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	155.47	0.00	155.47	11.213	108.70	119.91	35.56
2	155.47	0.00	155.47	12.966	111.23	124.20	31.27
3	155.47	40.22	195.69	21.877	137.78	159.66	36.03
4	155.47	42.76	198.23	26.982	132.17	159.15	39.08
5	155.47	38.25	193.72	22.042	142.44	164.48	29.24
6	155.47	42.17	197.64	24.072	144.57	168.64	29.00
7	155.47	8.58	164.05	17.865	116.84	134.70	29.35
8	155.47	15.50	170.97	22.371	116.52	138.89	32.08
9	155.47	48.85	204.32	26.469	138.64	165.11	39.21
10	155.47	55.77	211.24	32.140	141.80	173.94	37.30
11	155.47	51.96	207.43	33.136	138.17	171.31	36.12
12	155.47	58.31	213.78	36.698	137.70	174.40	39.38
13	155.47	46.83	202.30	25.836	150.10	175.94	26.36
14	155.47	53.80	209.27	30.297	149.86	180.16	29.11
15	155.47	50.75	206.22	26.964	147.50	174.46	31.76
16	155.47	57.72	213.19	32.457	147.97	180.43	32.76
17	155.47	16.13	171.60	19.584	124.58	144.16	27.44
18	155.47	15.60	171.07	18.736	123.40	142.14	28.93
19	155.47	54.31	209.78	28.004	156.66	184.66	25.12
20	155.47	53.83	209.30	26.777	152.86	179.64	29.66
Average.....	155.47	41.74*	193.04	24.824	135.97	160.80	32.24

* Average of 18.

down in pasture or receives an annual dressing of farmyard manure, the nitrogen in that soil will only increase so long as the annual increment of organic matter exceeds the annual decrement by oxidation. If this increment is a limited quantity it will be met before long with an army of destroyers competent to effect its destruction. The richest soils are thus the most liable to waste and demand the greatest exercise of the farmer's skill to preserve their condition.

When the conditions of the soil are changed, when the pasture is plowed up or the arable land is left without manure, there is at first a rapid loss of soil nitrogen, but the rate of loss soon diminishes. The organic matter most easily attacked has disappeared. The army of

oxidizing organisms has been reduced by starvation. A partial equilibrium is established when the annual destruction of organic matter amounts to little more than the annual residue of crop and weeds; but an absolute equilibrium is reached only when the annual loss of nitrogen is equaled by the atmospheric supply. In every case nature seeks to establish an equilibrium.

Russell (7) expresses about the same thought when he says:

Unfortunately on our present knowledge it is impossible to maintain a high content of nitrogen on cultivated land except at a wasteful expenditure of nitrogenous manure.

In discussing the loss of nitrogen from Rothamsted soils during a period of 50 years of cropping, Dyer (3) cites an annual balance of nitrogen unaccounted for amounting to nearly 70 pounds per acre on plots that received annually applications of ammonium salts equivalent to 86 pounds of nitrogen per acre. Dyer believed that this loss was largely in the form of nitrates that passed into the drains. He says:

Actual analyses of the drainage waters running from the pipes showed quantities of nitrates which go far toward accounting for the lost nitrogen, but the drain pipes only discharge a fraction of the total drainage, and a large quantity of drainage must often find its way downward below the level of the drainpipes when the pipes are not running, and even when they are.

Most, if not all, of the loss on the chemically manured plats may therefore, be said to be in the form of nitrates. It is possible that under certain conditions of weather there may be slight loss by evolution of free nitrogen from the crop residues of the surface soil on these plats but there is no evidence to show that any of the nitrogen added in the form of chemical manures is thus lost. But fermentative decomposition involving evolution of free nitrogen no doubt takes place on the heavily and continuously dunged plats, with their high quantity of organic matter and in a less degree on the rape-cake plat. It is to be borne in mind, however, that the quantity of dung used in these continuous wheat growing experiments is, on the yearly average, far less than would be used in practical agriculture on any of the rotation systems.

With reference to the exhaustion of the nitrogen supply of soils under continuous wheat growing, Dyer (3) says:

For the wheat crop has completed its growth and is harvested long before nitrification has ceased in the soil, and there is no crop to take up either the balance of the nitrates left unused by the wheat or the larger balance formed after the active growth of the wheat is over; and these nitrates must pass away in subsoil drainage during the winter, except when the rainfall is small, or in those latitudes exposed to long winter frosts. This drainage, even on unmanured land, involves a serious loss of nitrogen, and a still greater loss when the yield of the crops is artificially increased by the use of nitrogenous manure.

Snyder (8) has called attention to the enormous losses of humus and nitrogen which take place in continuous wheat growing in Minnesota. He writes:

One of the chief causes of the decline of fertility of old grain soils, is this loss of nitrogen by the rapid decay of the animal and vegetable matter of the soil, which has been accelerated by the continuous cultivation of grain. Thus of the 1700 pounds of nitrogen lost during the eight years of continuous wheat cultivation, 300 pounds only have been utilized by the wheat

as plant-food and 1400 pounds have been lost by the decay of the humus and the conversion of the nitrogen from the insoluble and stable humic forms to gaseous and soluble forms which are readily lost.

When wheat was grown continuously there was an annual loss of over 2000 pounds per acre of humus, due to the fermentation and decay of the animal and vegetable matter of the soil. When wheat was grown in a rotation with clover and oats, no material loss of humus from the soil occurred. When the nitrogen and humus of the soil were conserved by the rotation of crops, and the production of clover, an increase of 20 bushels per acre of corn and 5.6 bushels of wheat were secured.

Lyon and Bizzell (6) have shown an average annual loss of nitrogen in the drainage waters from three soil tanks kept free from vegetation amounting to 92 pounds per acre. The average loss through drainage and the removal of crops, from seven cropped tanks (no clover), during the same period, was 70 pounds per acre.

These tanks, both the cropped and those kept bare, received during the 5 years, two applications of farm manure amounting to 10 tons per acre each, which furnished a total of approximately 200 pounds of nitrogen per acre for the 5 years, or 40 pounds per year. No estimate was made of the amount of nitrogen lost through volatilization of gaseous nitrogen or ammonia.

It would thus appear that the soil in these tanks is losing nitrogen at the rate of 30 to 52 pounds per acre annually, aside from any loss that may occur through the volatilization of gaseous nitrogen or ammonia.

By the analysis of samples of soil from large drainage tanks in which orange trees were growing, Collison and Walker (2) have shown an average loss of nitrogen from three fertilized tanks (tank no. 2 is omitted inasmuch as a legume crop was grown on this) amounting to 525 pounds per acre for a period of 5 years, or an average annual loss of 105 pounds per acre. This loss was arrived at by noting the difference between the nitrogen content of the original soil and its content at the end of the 5-year period. It therefore includes loss through drainage waters, any nitrogen that the growing orange tree may have utilized and any loss through the volatilization of gaseous nitrogen or ammonia. Unfortunately, the report does not state the amount of nitrogen that was applied during the 5 years nor the amount of nitrogen that was lost in the *drainage* waters.

The conditions which obtain in the majority of our cylinders, and in the lysimeter experiments referred to are just the conditions which Russell says favor the decomposition of organic matter and the disappearance of nitrogen: namely, copious aeration (which implies good drainage) and the presence of large quantities of organic matter.

LOSS OF NITROGEN FOR THE SECOND TEN-YEAR PERIOD

The loss of nitrogen for the third 5-year period (calculated separately for the three cylinders of each series) is shown in table 2. It is a striking coincidence that the average loss for the *A*'s and *B*'s for the 5 years is just half of

TABLE 2

Loss of nitrogen during the third rotation

SERIES	*a* NITROGEN PRESENT, 1907	*b* NITROGEN APPLIED, 1908–1912	*m* $(a+b)$	*c* NITROGEN REMOVED BY CROPS 1908–1912	*d* NITROGEN PRESENT, 1912	*n* $(c+d)$	$(m-n)$ NITROGEN LOSS, EXCLUSIVE OF CROPS 1908–1912
			Section A				
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	109.26	0.00	109.26	2.62	93.46	96.08	13.18
2	107.60	0.00	107.60	4.11	90.46	94.57	13.03
3	124.98	20.00	144.98	9.04	118.66	127.70	17.28
4	128.69	20.00	148.69	9.75	133.75	143.50	5.19
5	144.49	20.00	164.49	9.27	133.35	142.62	21.87
6	146.55	20.00	166.55	9.97	144.81	154.78	11.77
7	120.63	3.87	124.50	6.17	106.18	112.35	12.15
8	114.71	7.71	122.42	9.27	104.52	113.79	8.63
9	136.59	23.87	160.46	11.75	131.69	143.44	17.02
10	144.81	27.71	172.52	14.58	131.69	146.27	26.25
11	142.20	23.87	166.07	13.12	130.59	143.71	22.36
12	136.59	27.71	164.30	14.23	128.14	142.37	21.93
13	146.15	23.87	170.02	11.52	137.38	148.90	21.12
14	144.25	27.71	171.96	14.29	138.72	153.01	18.95
15	146.31	23.87	170.18	12.78	142.60	155.38	14.80
16	146.86	27.71	174.57	14.99	157.84	172.83	1.74
17	123.87	8.18	132.05	6.58	104.91	111.49	20.56
18	124.19	8.42	132.61	7.33	117.00	124.33	8.28
19	155.00	28.18	183.18	13.62	146.47	160.09	23.09
20	151.36	28.42	179.78	13.27	144.41	157.68	22.10
Average.....	134.75		153.31	10.41	126.83	137.24	16.06
			Section B				
1	107.60	0.00	107.60	4.28	91.80	96.02	11.58
2	109.81	0.00	109.81	5.72	89.27	94.99	14.82
3	139.20	20.00	159.20	11.23	127.66	138.89	20.31
4	133.98	20.00	153.98	11.30	121.58	132.88	21.10
5	135.88	20.00	155.88	10.38	136.75	147.13	8.75
6	144.81	20.00	164.81	11.34	137.07	148.41	16.40
7	113.76	3.87	117.63	7.97	104.52	112.49	5.14
8	114.08	7.71	121.79	11.07	103.49	114.56	7.23
9	133.83	23.87	157.70	13.57	129.24	142.81	14.89
10	135.88	27.71	163.59	16.43	133.91	150.34	13.25
11	135.88	23.87	159.75	16.35	125.06	141.41	18.34
12	131.40	27.71	159.11	16.49	126.95	143.44	15.67
13	145.60	23.87	169.47	15.02	132.40	147.42	22.05
14	155.71	27.71	183.42	15.30	139.99	155.29	28.13
15	146.15	23.87	170.02	13.87	134.85	148.72	21.30
16	144.49	27.71	172.20	16.41	137.62	154.03	18.17
17	124.27	8.18	132.45	9.28	111.47	120.75	11.70
18	118.26	8.42	126.68	8.74	112.26	121.00	5.68
19	158.47	28.18	186.65	15.24	149.07	164.31	22.34
20	153.66	28.42	182.08	15.60	139.75	155.35	26.73
Average.....	134.14		152.69	12.28	124.24	136.51	16.18

TABLE 2—*Continued*

Section C

SERIES	*a* NITROGEN PRESENT, 1907	*b* NITROGEN APPLIED, 1908-1912	*m* $(a+b)$	*c* NITROGEN REMOVED BY CROPS, 1908-1912	*d* NITROGEN PRESENT 1912	*n* $(c+d)$	$(m-n)$ NITROGEN LOSS, EXCLUSIVE OF CROPS 1908-1912	A's, B's, C's Average amount of nitrogen applied	Average amount of nitrogen lost
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	109.26	0.00	109.26	5.59	92.90	98.49	10.77	0.00	11.84
2	116.13	0.00	116.13	7.24	102.30	109.54	6.59	0.00	11.48
3	149.15	20.00	169.15	12.09	136.28	148.37	20.78	20.00	19.46
4	133.83	20.00	153.83	12.92	140.38	153.30	0.53	20.00	8.94
5	146.86	20.00	166.86	14.59	150.10	164.69	2.17	20.00	10.93
6	142.44	20.00	162.44	14.07	138.49	152.56	9.88	20.00	12.68
7	116.13	3.87	120.00	9.56	106.49	116.05	3.95	3.87	7.08
8	120.87	7.71	128.58	12.43	109.57	122.00	6.58	7.71	7.48
9	145.44	23.87	169.31	17.22	142.12	159.34	9.97	23.87	13.96
10	144.81	27.71	172.52	18.87	142.12	160.99	11.53	27.71	17.01
11	137.14	23.87	161.01	16.91	134.46	151.37	9.64	23.87	16.78
12	145.44	27.71	173.15	20.41	140.54	160.95	12.20	27.71	16.60
13	158.47	23.87	182.34	16.76	145.20	161.96	20.38	23.87	21.18
14	149.55	27.71	177.26	18.08	145.99	164.07	13.19	27.71	20.09
15	150.81	23.87	174.68	17.15	146.55	163.70	10.98	23.87	15.69
16	152.47	27.71	180.18	17.82	146.94	164.76	15.42	27.71	11.78
17	125.69	8.18	133.87	10.84	118.66	129.50	4.37	8.18	12.21
18	127.74	8.42	136.16	10.26	115.89	126.15	10.01	8.42	7.99
19	156.42	28.18	184.60	17.18	156.02	173.20	11.40	28.18	18.94
20	153.66	28.42	182.08	18.51	143.23	161.74	20.34	28.42	23.06
Average...	139.12		157.67	14.43	132.71	147.14	10.534		14.26

the average loss for the first 10 years, that is, the annual rate of loss for the third 5 years is exactly the same as for the first 10 years, namely 103 pounds per acre.

The *apparent* average loss for the *C*'s is less by a little more than one-third. The *apparent* loss is referred to here for the reason that these are the cylinders on which a legume is grown for green manure, and they therefore have at their disposal a certain amount of nitrogen in excess of the amount at the disposal of the *A*'s and *B*'s which amount cannot be definitely measured. For this reason, the figures, which are obtained by an indirect process, probably do not represent the full amount of the loss.

For this period those cylinders that receive the smaller applications of nitrogen generally show less loss than those that receive the heavy applications. The greatest loss, in most cases, is sustained by those cylinders that receive the manure or the manure and nitrate in combination.

The data for the fourth 5-year period are shown in table 3. Three out of the 20*A* cylinders made slight gains. There appears to be no particular reason why the three cylinders should have made gains when all the others

TABLE 3

Loss of nitrogen during the fourth rotation

SERIES	*a* PRESENT 1913	*b* APPLIED 1913-1917	*m* $(a+b)$ TOTAL PRESENT AND APPLIED	*c* REMOVED 1913-1917	*d* PRESENT 1917	*n* $(c+d)$ TOTAL REMOVED AND PRESENT	$m-n$ LOSS EXCLUSIVE OF CROPS 1913-1917
			Section A				
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	93.46	0.00	93.46	1.75	87.30	89.05	4.41
2	90.46	0.00	90.46	2.39	83.32	84.71	5.75
3	118.66	20.06	138.72	8.95	119.69	128.64	10.08
4	133.75	20.06	153.81	9.72	120.08	129.80	24.01
5	133.35	20.06	153.41	8.51	131.46	139.97	13.44
6	144.81	20.06	164.87	9.10	140.23	149.33	15.54
7	106.18	3.85	110.03	4.16	94.01	98.17	11.86
8	104.52	7.70	112.22	5.07	101.04	106.11	6.11
9	131.69	23.91	155.60	9.95	150.42	160.37	4.77‡
10	131.69	27.76	159.45	12.65	143.54	156.19	3.26
11	130.59	23.91	154.50	12.53	132.48	145.01	9.49
12	128.14	27.76	155.90	13.68	142.44	156.12	0.22‡
13	137.38	23.91	161.29	10.25	144.73	154.98	6.31
14	138.72	27.76	166.48	13.23	153.81	167.04	0.56‡
15	142.60	23.91	166.51	11.90	150.34	162.24	4.27
16	157.84	27.76	185.60	14.23	157.53	171.76	13.84
17	104.91	7.70	112.61	4.46	87.45	91.91	20.70
18	117.00	7.70	124.70	5.08	104.68	109.76	14.94
19	146.47	27.76	174.23	12.00	148.60	160.60	13.63
20	144.41	27.76	172.17	10.88	144.02	154.90	17.27
Average......	126.83	20.52*	145.30	9.03	126.81	135.83	11.46†
			Section B				
1	91.80	0.00	91.80	4.39	82.32	86.71	5.09
2	89.27	0.00	89.27	4.77	83.90	88.67	0.60
3	127.66	20.06	147.72	10.14	124.19	134.33	13.39
4	121.58	20.06	141.64	11.86	120.00	131.86	9.78
5	136.75	20.06	156.81	10.38	128.77	139.15	17.66
6	137.07	20.06	157.13	11.01	142.28	153.29	3.84
7	104.52	3.85	108.37	7.40	92.67	100.07	8.30
8	103.49	7.70	111.19	9.33	94.09	103.42	7.77
9	129.24	23.91	153.15	11.78	134.40	146.18	6.97
10	133.91	27.76	161.67	14.12	138.41	152.53	9.14
11	125.06	23.91	148.97	15.26	124.19	139.45	9.52
12	126.95	27.76	154.71	14.96	129.17	144.13	10.58
13	132.40	23.91	156.31	12.87	133.98	146.85	9.46
14	139.99	27.76	167.75	13.23	141.57	154.80	12.95
15	134.85	23.91	158.76	12.77	142.60	155.37	3.39
16	137.62	27.76	165.38	14.30	136.99	151.29	14.09
17	111.47	7.70	119.17	8.39	104.91	113.30	5.87
18	112.26	7.70	119.96	7.77	104.12	111.89	8.07
19	149.07	27.76	176.83	12.57	140.94	153.51	23.32
20	139.75	27.76	167.51	13.78	140.07	153.85	13.66
Average......	124.24	20.52*	142.71	11.05	121.98	133.03	9.67

* Average of 18 determinations.

‡ Gain.

† Average of 17 determinations.

TABLE 3—*Continued*

SERIES	*a* PRESENT 1913	*b* APPLIED 1913-1917	*m* $(a+b)$ TOTAL PRESENT AND APPLIED	*c* REMOVED 1913-1917	*d* PRESENT, 1917	*n* $(c+d)$ TOTAL REMOVED AND PRESENT	*m — n* EXCLUSIVE OF CROPS Loss	Gain
				Section C				
	gm.	gm.	gm.	gm.	gm.	gm	gm.	gm.
1	92.90	0.00	92.90	6.67	90.69	97.36		4.46
2	102.30	0.00	102.30	8.96	97.96	106.92		4.62
3	136.28	20.06	156.34	13.41	143.07	156.48		0.14
4	140.38	20.06	160.44	15.03	134.70	149.73	10.71	
5	150.10	20.06	170.16	14.35	144.73	159.08	11.08	
6	138.49	20.06	158.55	15.61	150.50	166.11		7.56
7	106.49	3.85	110.34	10.97	107.91	118.88		8.54
8	109.57	7.70	117.27	12.74	99.62	112.36	4.91	
9	142.12	23.91	166.03	15.42	145.52	160.94	5.09	
10	142.12	27.76	169.88	16.51	142.44	158.95	10.93	
11	134.46	23.91	158.37	16.86	136.35	153.21	5.16	
12	140.54	27.76	168.30	20.16	141.25	161.41	6.89	
13	145.20	23.91	169.11	14.69	151.60	166.29	2.82	
14	145.99	27.76	173.75	17.10	162.50	179.60		5.85
15	146.55	23.91	170.46	16.19	157.45	173.64		3.18
16	146.94	27.76	174.70	17.40	151.13	168.53	6.17	
17	118.66	7.70	126.36	11.37	115.74	127.11		0.75
18	115.89	7.70	123.59	11.87	107.20	119.07	4.52	
19	156.02	27.76	183.78	16.86	156.97	173.83	9.95	
20	143.23	27.76	170.99	17.53	162.90	180.43		9.44
Average......	132.71	20.52*	151.18	14.49	135.01	149.50	7.11†	

* Average of 18 determinations.
† Average of 11 determinations.

were losing. The average loss for the remaining seventeen is 11.46 gm. equivalent to 73.3 pounds per acre, or approximately two-thirds of the amount lost during the third 5 years.

A loss is shown for all of the *B*'s for the fourth period, though in the case of 2*B* it is almost negligible. It would appear that here, too, the nitrogen equilibrium, of which Russell speaks, has been reached. The heaviest loss—23.32 gm.—occurs with 19*B* which receives manure and ammonium sulfate. All cylinders from 9*B* to 16*B*, inclusive, receive like amounts of nitrogen in cow manure, and in addition the odd numbers receive 5 gm. of nitrate of soda and the even numbers 10 gm., and in this connection it may be pointed out that the latter show a greater loss than the former.

The average loss for the entire series is 9.67 gm. equivalent to a yearly average of 61.9 pounds per acre. Of the *C*'s 11 show losses averaging 7.11 gm. and 9 show gains averaging 4.99 gm. In the case of 3*C* and 17*C* the gains are only slight and here again an equilibrium appears to have been reached.

As previously explained these gains are apparent rather than real for the reason that an unknown amount of atmospheric nitrogen has been supplied by the two legume crops, and this has not been included in the nitrogen applied.

PERCENTAGE OF NITROGEN IN SOILS

In connection with the foregoing discussion it is of interest to study the figures showing the percentage of nitrogen in the original soil used in these cylinders, in comparison with the composition of samples drawn from the various cylinders at 5-year intervals. These figures are shown in table 4. On account of the difference in treatment during the second 10-year period it is necessary to report the *A*, *B*, and *C* sections separately. It will be noted that in most cases these figures show a general decline in nitrogen content. In a few cases, notably 14*A*, 15*A* and 16*A*, the nitrogen content has been kept well up to the original condition. The same is true of three or four of the cylinders in section *C*, but on account of the legume crops which were grown in this section, they cannot fairly be considered in connection with the two other sections.

The rather rapid decline in nitrogen content of series 1 and 2 is not surprising. These cylinders, it will be remembered, produce crops each year without having any nitrogen added. In the 20 years then, the nitrogen content has dropped from nearly 0.2 per cent to about 0.10 to 0.11 per cent. No. 7, 8, 17 and 18 also have lost heavily. These are the cylinders that receive all their nitrogen in the form of commercial fertilizers and do not receive any farm manure, and with the exception of those in section *C*, do not receive any green manure.

This is a good illustration of what happens when land is continually under cultivation without the use of green-manure crops or farm manures to aid in maintaining the supply of organic matter. Even the green-manure crop cylinders show a rather heavy loss.

It may be pointed out that during the period of 1912 to 1918 the losses were not very great in most cases. Indeed in some cases there were fair increases in nitrogen content. Taking the averages for all the series it is found that the nitrogen content remained very nearly constant during this period. This must not be taken as a contradiction of results reported in table 3 where a loss of nitrogen is shown for this same period, for it must be remembered that nearly twice as much nitrogen was applied as was removed by the crops during the 5 years.

It is possible that the soil in series 1 and 2 has reached an equilibrium which may be maintained for a number of years, though this could not go on indefinitely if the land is continuously cropped without the use of nitrogenous fertilizers or green manures.

It is of interest to note that the soils of section *B* show a lower nitrogen content than those of either of the other sections.

TABLE 4

Percentage of nitrogen present, air-dry soil—1898, 1907, 1912, 1918

SERIES	SECTION A				SECTION B				SECTION C			
	Nitrogen originally present	Present in 1907	Present in 1912	Present in 1918	Originally present	Present in 1907	Present in 1912	Present in 1918	Originally present	Present in 1907	Present in 1912	Present in 1918
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	0.1968	0.1383	0.1183	0.1105	0.1968	0.1362	0.1162	0.1042	0.1968	0.1383	0.1176	0.1148
2	0.1968	0.1362	0.1145	0.1042	0.1968	0.1390	0.1130	0.1062	0.1968	0.1470	0.1295	0.1240
3	0.1968	0.1582	0.1502	0.1515	0.1968	0.1762	0.1616	0.1572	0.1968	0.1888	0.1725	0.1811
4	0.1968	0.1629	0.1693	0.1520	0.1968	0.1696	0.1539	0.1519	0.1968	0.1694	0.1777	0.1705
5	0.1968	0.1829	0.1688	0.1664	0.1968	0.1720	0.1731	0.1630	0.1968	0.1859	0.1900	0.183[illegible]
6	0.1968	0.1855	0.1833	0.1775	0.1968	0.1833	0.1735	0.1801	0.1968	0.1803	0.1753	0.19[illegible]
7	0.1968	0.1527	0.1344	0.1190	0.1968	0.1440	0.1323	0.1173	0.1968	0.1470	0.1348	0.13[illegible]
8	0.1968	0.1452	0.1323	0.1279	0.1968	0.1444	0.1310	0.1191	0.1968	0.1530	0.1387	0.12[illegible]
9	0.1968	0.1729	0.1667	0.1904	0.1968	0.1694	0.1636	0.1701	0.1968	0.1841	0.1799	0.184
10	0.1968	0.1833	0.1667	0.1817	0.1968	0.1720	0.1695	0.1752	0.1968	0.1833	0.1799	0.18[illegible]
11	0.1968	0.1800	0.1653	0.1677	0.1968	0.1720	0.1583	0.1572	0.1968	0.1736	0.1702	0.172
12	0.1968	0.1729	0.1622	0.1803	0.1968	0.1660	0.1607	0.1635	0.1968	0.1841	0.1779	0.1788
13	0.1968	0.1850	0.1739	0.1832	0.1968	0.1843	0.1676	0.1696	0.1968	0.2006	0.1838	0.1919
14	0.1968	0.1826	0.1756	0.1947	0.1968	0.1971	0.1772	0.1792	0.1968	0.1893	0.1848	0.2057
15	0.1968	0.1852	0.1805	0.1903	0.1968	0.1850	0.1707	0.1805	0.1968	0.1909	0.1855	0.1993
16	0.1968	0.1859	0.1998	0.1994	0.1968	0.1829	0.1742	0.1734	0.1968	0.1930	0.1860	0.1913
17	0.1968	0.1568	0.1328	0.1107	0.1968	0.1573	0.1411	0.1328	0.1968	0.1591	0.1502	0.1465
18	0.1968	0.1572	0.1481	0.1325	0.1968	0.1497	0.1421	0.1318	0.1968	0.1617	0.1467	0.1357
19	0.1968	0.1962	0.1854	0.1881	0.1968	0.2006	0.1887	0.1784	0.1968	0.1980	0.1975	0.1987
20	0.1968	0.1916	0.1828	0.1823	0.1968	0.1945	0.1769	0.1773	0.1968	0.1945	0.1813	0.2062
Average......	0.1968	0.1706	0.1605	0.1605	0.1968	0.1698	0.1573	0.1544	0.1968	0.1761	0.1680	0.1709

This should probably be attributed to the more rapid oxidation of the organic matter in the presence of lime. Lime was applied also to section *C* but the legume green-manure crops that are grown on this section have tended to raise the nitrogen content above that of the other two sections.

THE CARBON CONTENT OF CYLINDER SOILS

Carbon determinations were made on the soils collected in 1918, and are reported in table 5. A comparison of the carbon content at this time, with

TABLE 5

Percentage of carbon in cylinder soils—1918

SERIES	SECTION A	SECTION B	SECTION C
	per cent	*per cent*	*per cent*
1	1.390	1.360	1.630
2	1.350	1.285	1.670
3	2.005	1.975	2.365
4	1.835	1.730	2.105
5	1.955	2.010	2.285
6	2.045	2.135	2.500
7	1.235	1.335	1.745
8	1.305	1.375	1.740
9	2.245	2.175	2.300
10	2.260	2.225	2.450
11	1.975	1.895	2.295
12	2.140	1.790	2.340
13	2.210	2.135	2.460
14	2.445	2.135	2.555
15	2.320	2.175	2.405
16	2.340	2.095	2.310
17	1.280	1.595	1.725
18	1.405	1.540	1.795
19	2.300	2.220	2.455
20	2.090	2.085	2.360
Average..........	1.907	1.864	2.175

the carbon content of the original soil, is instructive. A determination made on a sample of the original soil showed 1.83 per cent of carbon.

It will be noted that in a number of cases the carbon content has increased, while in a few cases it has decreased. Those cylinders that have received manure alone have increased but slightly in carbon content; those that have received both manure and nitrate of soda, in most cases have made considerable gains. In section *A* those that receive the 10-gm. portion of nitrate—no. 10, 12, 14 and 16—have made greater gains than those that receive the 5-gm. portion—no. 9, 11, 13 and 15.

Those in sections *A* and *B* that receive no nitrogen and also those that receive commercial nitrogenous materials only, have lost notably. On section *C* the green-manure crop has aided in keeping up the carbon supply.

Attention may be called to the fact that the average carbon content of the soils in section *B* is less than the average in section *A*.

This would seem to be further evidence that the lime favored the oxidation and disappearance of the organic matter, while the higher average on section *C* shows the value of a green-manure crop in maintaining the supply of organic matter.

There need not necessarily be any inconsistency in an increase in the carbon content of a soil, the nitrogen content of which is decreasing. With the decomposition of the less resistant part of the organic matter which contains much of the nitrogen, there may be an accumulation in the soil of the harder more resistant parts of the plant residues, thus increasing the carbon content.

SUMMARY

A study has been made of nitrogen losses from a loam soil (in cylinders with natural drainage) which has been under a 5-year rotation of corn, oats (2 years), wheat and timothy, for 20 years.

There are 60 of these cylinders arranged in 20 series of 3 cylinders each, and the fertilizer and manure treatment is the same for the 3 cylinders of a given series.

Different forms of nitrogenous fertilizers are used alone and also in combination with farm manure. All cylinders with the exception of those of series 1, receive liberal applications of phosphoric acid and potash annually.

During the first 10 years, the lime treatment was the same for all cylinders. Beginning with the second 10-year period, lime (ground limestone) has been applied to the second and third cylinders of each series at 5-year intervals. A further differentiation has been brought about by growing two legume green-manure crops on the third cylinder of each series in the course of each 5-year rotation. Thus the first cylinder of each series, or 20 cylinders designated as section *A*, have been without lime for 20 years.

A complete record has been kept of the amount of nitrogen applied in the form of fertilizers or manures, and also of the amount removed by the crops during this period. Also, nitrogen determinations have been made on samples of the original soil and on samples collected in 1907, 1912 and 1918. From these latter records, the loss of nitrogen from the soil, exclusive of that removed by the crops, has been calculated.

During the first 10 years, this amounted to 32.24 gm. per cylinder, or the equivalent of 103 pounds per acre annually. During the third 5-year period, the loss was one-half this amount (except in the legume section), or again 103 pounds per acre annually. During the fourth 5-year period, the average loss was considerably less, and for certain of the cylinders on sections *A* and *C* there was some gain. This gain on section *C* is due largely to the legume

crops that were grown on this section. There appears to be no particular reason for the gain on three of the cylinders of section *A*. This statement of gain, however, is not a contradiction of the statement that in most cases these soils have been constantly losing in nitrogen content, since with only one or two exceptions more nitrogen has been applied than has been removed by the

Carbon determinations made on the set of samples collected in 1918 show gains in carbon content over the original soil, for nearly all those cylinders that receive both farm manure and commercial nitrogenous fertilizers. Those soils that receive farm manure alone have not been changed much in carbon content, while those that have received nitrogen in the form of commercial fertilizers only, or have received no nitrogen at all have lost appreciably in carbon content.

A gain in carbon is not necessarily inconsistent with a loss in nitrogen.

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PLATE 1

Fig. 1. Wheat on Nitrate of Soda Cylinders (Series 8), 1916
Compare *A* (no lime) with *B* and *C* (limed)

Fig. 2. Wheat on Sulfate of Ammonia Cylinders (Series 17), 1916

The continued use of sulfate of ammonia without lime has rendered soil practically sterile. Compare *A* with *B* and *C*.

Fig. 3. Wheat on Manure and Sulfate of Ammonia Cylinders (Series 19), 1916

Manure appears to aid in overcoming the unfavorable condition produced by sulfate of ammonia when used alone; compare *A* with *A* in figure 2.

Note: A's unlimed; *B's* and *C's* limed at intervals of 5 years; *C's* produce two legume green-manure crops every 5 years.

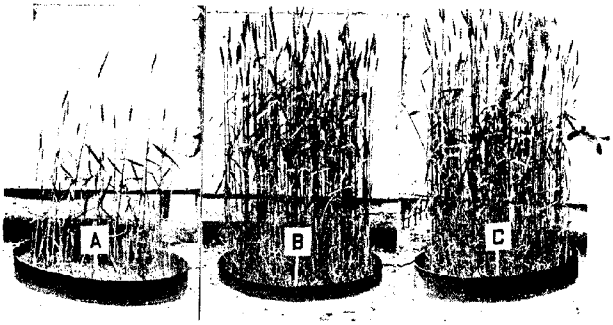

FIG. 1

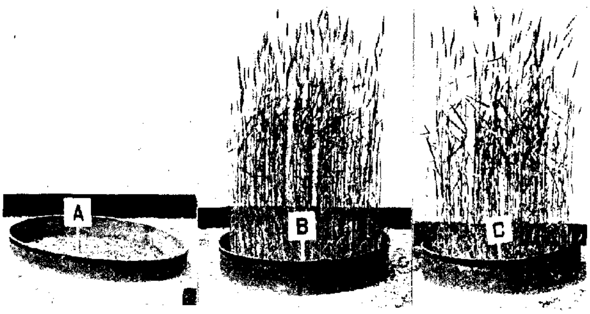

FIG. 2

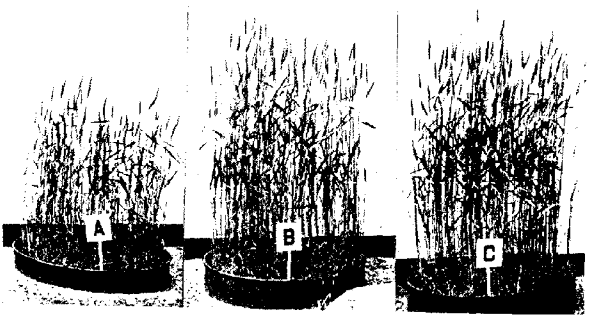

FIG. 3

PLATE 2

FIG. 1. TIMOTHY ON "NO FERTILIZER" CYLINDERS (SERIES 1), 1917

20 years of cropping without fertilizer or lime has left the soil without producing power; compare *A* with *B* and *C*.

FIG. 2. TIMOTHY ON CHECK CYLINDERS (SERIES 2), 1917

Minerals annually, but no nitrogen applied; nitrogen long ago became the limiting factor (see *A*), but when lime is applied a moderate crop is still produced (see *B* and *C*).

FIG. 3. TIMOTHY ON MANURE CYLINDERS (SERIES 3), 1917

Manure supplies the needed nitrogen and appears also to satisfy the need for lime; compare *A* with *B* and *C*.

Note: A's unlimed; *B's* and *C's* limed at intervals of 5 years; *C's* produce two legume green-manure crops every 5 years.

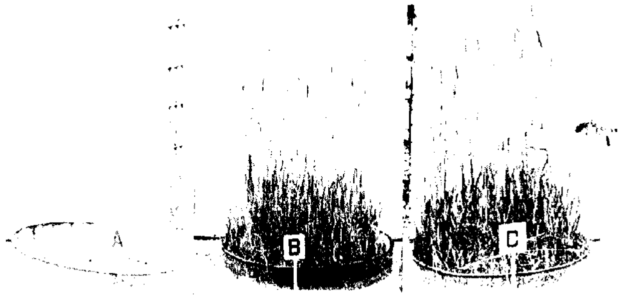

FIG. 1

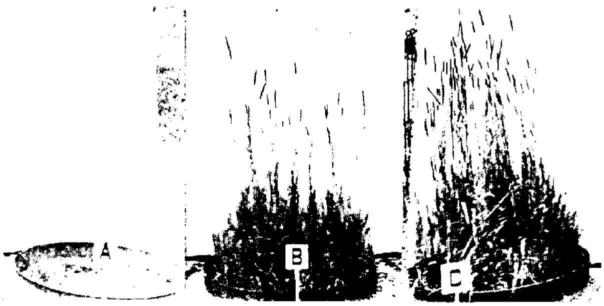

FIG. 2

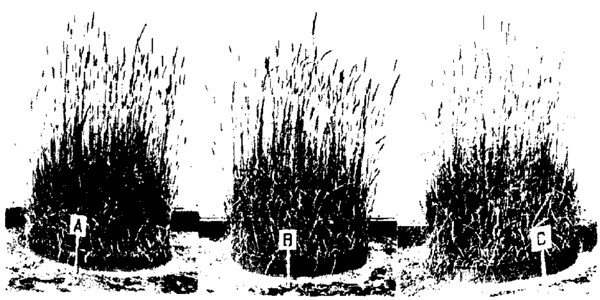

FIG. 3

THE RELATION OF ORGANIC MATTER AND THE FEEDING POWER OF PLANTS TO THE UTILIZATION OF ROCK PHOSPHATE[1]

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The processes accompanying the decomposition of organic matter and the feeding power of plants are undoubtedly important factors in the utilization of rock phosphate. Those who strongly advocate the use of rock phosphate for soil improvement always advise that it be applied in intimate contact with decaying organic matter (13). The explanation usually given for this practice is that the organic acids, carbon dioxide and nitrous acid, resulting from such decomposition, are active agents in making the phosphate available. Strangely, however, when attempts have been made in the laboratory (12, 22, 31, 42) to prove this explanation, positive results have usually not been obtained, although in field tests rock phosphate has usually given the best results when used in connection with considerable organic matter. On the other hand, many investigators (5, 15, 17, 21, 24, 25, 28, 32, 33, 34, 37, 38) have observed that the growing plant itself possesses more or less power to feed directly on rock phosphate and that some plants possess especially marked powers. As a rule it has been found that most of the cereals are among the poor feeders, while the legumes, cruciferae and buckwheat are among the good feeders. The possibility of planning systems of farming in which the strong feeders may be used advantageously for an effective utilization of rock phosphate is of much practical importance.

HISTORICAL REVIEW

In explanation of the difficulty of showing that decaying organic matter does made rock phosphate available Truog (42) suggested that the particles of rock phosphate and also the particles of the finely divided reprecipitated phosphate may be protected by slimy films of organic matter from extraction by the methods employed in the laboratory. He stated further that laboratory

[1] Part II of thesis submitted at the University of Wisconsin in partial fulfillment of the requirements for the Degree of Doctor of Philosophy. Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

The writer wishes to express his appreciation for the helpful suggestions and criticisms tendered by Professor E. Truog.

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composting experiments do not imitate field conditions and thus the efficiency of the carbon dioxide as a solvent may be lowered. He concluded that the decay of organic matter does increase the availability of rock phosphate by favoring a more thorough mechanical distribution of the phosphate in the soil, and the biological processes that give rise to the formation of carbon dioxide.

Tottingham and Hoffman (41) found that the amount of water-soluble phosphorus in fermenting mixtures of manure and rock phosphate decreased over periods of 4 to 6 months. They attributed this decrease, in part at least, to the use of soluble phosphorus by the bacteria themselves. They concluded that the final results from the mixing of manure and rock phosphate are advantageous.

Hopkins and Whiting (14) found that nitrite bacteria have the power of oxidizing ammonium sulfate in solution cultures containing pure tri-calcium phosphate, but no carbonate or free base, and that the nitrous and sulfuric acids resulting bring phosphorus into solution. The process is not accompanied with changes in acidity. They suggested the possibility of similar effects in the soil resulting from the decomposition of nitrogenous organic matter.

Truog (42) found that fermenting mixtures of manure and June grass became more alkaline as decomposition proceeded and pointed out that acids formed from decaying organic matter are likely to be neutralized before they could affect the rock phosphate.

Kelley (16) found that the calcium of rock phosphate does not furnish the calcium for nitrification under soil conditions and concluded that the decomposition of organic matter from the standpoint of nitrification has little effect on the availability of rock phosphate.

The earlier views held that plants excrete acids from their roots, which in themselves are the effective agents in the solution of mineral nutrients, like phosphates. In 1902 Kossowitsch (18) performed his famous experiment showing that the plant roots must be in contact with the rock phosphate in order to secure the phosphorus. Dyer (10), Quartaroli (36), Pfeiffer and Blanck (30), Palladin (27) and Maze (23) expressed their belief in the acid excretion theory. Shulov (39) found that ammonium nitrate favored the excretion of malic acid from plant roots, and thus brought rock phosphate into solution. Carso (6), though not stating definitely that acids are excreted from plant roots, did claim that the feeding power of plants of the same family for rock phosphate varies to some extent according to the acid content of the plant. Bogdanov (4), Prianischnikov (35), Chirikov (8), and Haas (11), however, claimed that no acids other than carbonic are excreted, and therefore no solvent power except that due to the carbon dioxide exists.

Kossowitsch (19, 20) and Stoklasa and Ernst (40) studied the carbon-dioxide excretion of roots of certain plants and found it to be quite large. Kossowitsch detected no relation between the feeding power of plants and the amount of carbon dioxide excreted. None of these experiments, however,

were carried out under natural and uniform conditions, and indicate a field for further research. Bogdanov (4) claimed that since the amount of carbon dioxide normally present in the soil is large the carbon dioxide excreted by plants would have but little additional solvent effect on rock phosphate. Truog (42), however, believed that the carbon dioxide excreted by the plant roots works at maximum efficiency in dissolving rock phosphate because of the removal of the soluble products. When carbon dioxide was used in culture experiments (26, 29, 45) only negative results on the solution of insoluble phosphates were secured.

In 1914 and 1916 Chirikov (7, 8) published the results of his experiments from which he concluded that plants which more energetically assimilate calcium are the better feeders on rock phosphate. He stated that when the ratio of CaO to P_2O_5 in the plant is greater than 3 the plant is usually a good feeder. In 1915 and 1916 Truog (43, 44) put forth the theory that it is the CaO content of the plant and not the ratio of CaO to P_2O_5 that bears an important relation to the feeding powers of plants.

EXPERIMENTAL

Materials used

The soil medium consisted of either sand or soil. The sand was practically pure white quartz and contained no soluble phosphorus. The soil was a strongly acid Plainfield sand, which in the air-dry condition contained 0.59 parts per million of water-soluble phosphorus.

The different forms of organic matter used consisted of finely pulverized air-dry buckwheat hay, sweet clover hay, alfalfa hay, soybean hay, mammoth clover hay, corn stover, oats straw, and chopped green buckwheat and sweet clover.

The rock phosphate used was a finely ground brown rock containing 15.4 per cent of total phosphorus, 0.025 per cent of water-soluble phosphorus and 0.076 per cent of phosphorus soluble in water saturated with carbon dioxide. The acid phosphate was a commercial product containing 6.5 per cent of phosphorus. The limestone was a pulverized dolomite containing a large amount of fine material. Unless otherwise specified these materials were used at the rate of 1 ton, 400 pounds and 2 tons to the acre, respectively.

The nutrient solution used in the culture experiments was the same as that employed in the experiments previously reported (3) in which $NaNO_3$ was used as the nitrogen salt. The pot cultures described herein were managed in the same manner as those in the experiments just referred to.

The effect of organic matter without leaching on the availability of rock phosphate

Manure has usually been employed as the form of organic matter in studies of this kind. Blood meal, June grass and cottonseed meal have also been used. No record was found of studies with other residues which a farmer

might use in this connection. Consequently some preliminary studies were made with sweet clover, mammoth clover and buckwheat hay.

The work was carried on in Mason fruit jars containing 500 gm. of either sand or soil. Applications in accordance with the arrangement indicated in table 1 were made as follows on the acre basis: organic matter 5 tons, rock phosphate 1 ton and limestone 2 tons. The soluble phosphorus was determined in both the organic matter and phosphate used. At the time of starting, 10 cc. of a filtered soil infusion was added to each jar. The moisture content was maintained at 13 per cent of the weight of the mixture. The mixtures were agitated frequently by vigorous shaking of the jars and allowed to remain in a dark place in the greenhouse. All mixtures were set up in duplicate.

TABLE 1

The amounts of water-soluble phosphorus in each jar before and after decomposition of organic matter, in mixtures indicated

FORMS OF ORGANIC MATTER	TREATMENTS	SAND MIXTURES			SOIL MIXTURES		
		Amount of soluble phosphorus		Decrease of soluble phosphorus	Amount of soluble phosphorus		Decrease of soluble phosphorus
		Beginning	End		Beginning	End	
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Sweet clover	None	5.45	1.90	3.55	5.74	1.90	3.84
	Rock phosphate	5.87	2.30	3.57	6.16	2.60	3.56
	Rock phosphate, lime	5.87	3.10	2.77	6.16	1.00	5.16
Mammoth clover	None	5.40	2.70	2.70	5.69	1.30	4.39
	Rock phosphate	5.82	3.00	2.82	6.11	1.20	4.91
	Rock phosphate, lime	5.82	2.60	3.22	6.11	1.00	5.11
Buckwheat	None	6.55	0.40	6.15	6.84	0.70	6.14
	Rock phosphate	6.97	3.75	3.22	7.26	0.75	6.51
	Rock phosphate, lime	6.97	2.20	4.77	7.26	0.80	6.46

At the end of nearly 5 months the mixtures were brought to an air-dry condition, by drying rapidly in an oven and then exposing to laboratory conditions before analysis. Samples of 200 gm. were shaken vigorously by hand with 500 cc. of distilled water, allowed to settle and the supernatant liquid filtered off through Pasteur-Chamberland clay filters. The filtered solutions, after oxidation with bromine water to destroy the organic matter, were analyzed for phosphorus by the Pemberton method.

The results are recorded in table 1. The amounts of soluble phosphorus found were not large in any case and did not show any regularity to the treatments given. There was usually a slight increase of soluble phosphorus where rock phosphate was used in addition to the organic matter. In some cases the limestone appeared to increase slightly the solubility of the phosphate,

while in others, it caused a slight decrease. In accord with the results of previous investigators, in no case was there found to be as much soluble phosphorus at the end of the decomposition period as there was known to exist at the beginning. With all combinations the soluble phosphorus became more or less insoluble.

The effect of organic matter with leaching on the availability of rock phosphate

In view of the failure of experiments to show that the decomposition of organic matter makes rock phosphate available, it was thought that perhaps leaching the mixtures once a week would better imitate the conditions of the field and thus show a solvent effect. Two series of percolators containing sweet clover hay as the organic matter and other materials similar in kind

TABLE 2

Water-soluble phosphorus, calcium and nitric nirogen in leachings from series leached at intervals and series leached only at the end

MEDIUM	TREATMENTS	SERIES LEACHED AT INTERVALS			SERIES LEACHED ONLY AT THE END		
		P	Ca	Nitric N	P	Ca	Nitric N
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Sand...	Phosphate	1.70	26.06	10.00	1.64	15.04	3.00
	Sweet clover	2.80	49.88	29.62	2.24	17.64	24.50
	Sweet clover, phosphate	2.30	46.90	18.62	1.82	16.84	26.50
	Sweet clover, phosphate, lime	2.32	45.90	20.60	1.70	17.84	24.50
Soil....	None	2.16	28.46	23.12	1.32	16.84	14.00
	Phosphate	2.20	28.46	23.60	1.32	18.85	15.50
	Sweet clover	2.26	25.84	31.10	0.98	35.89	49.00
	Sweet clover, phosphate	2.34	25.66	29.18	1.52	36.29	47.50
	Sweet clover, phosphate, lime	1.76	31.16	39.28	1.28	42.71	62.00

and quantity to those in the experiments just reported were arranged. One series was leached weekly with 200 cc. of distilled water for a period of 12 weeks, and the other only at the end. Each treatment was duplicated. The inoculated mixtures were kept in a dark place in the greenhouse and stirred and watered as needed. The leachings were filtered immediately through Pasteur-Chamberland clay filters. After a period of more than 3 months, the other series was leached with 200-cc. portions of distilled water until a total of 1200 cc. of leachings were secured. These were filtered in the same manner as the others. The filtered solutions from both series were analyzed for phosphorus, calcium, and nitric nitrogen. The phosphorus and calcium were determined volumetrically by the Pemberton and permanganate methods, respectively, after the organic matter in solution had been decomposed by bromine water. The nitric nitrogen was determined by Devarda's reduction method.

The results are given in table 2. The data of the two series are not strictly comparable on account of the fact that the total leachings from the series leached at intervals was twice as large as from the other. The amount of soluble phosphorus was not large in either series. There was but little difference between the influence of the quartz sand and of the Plainfield sand. The soluble calcium in the regularily leached series was higher in the sand than in the soil, but in the other series this was not true. Nitrification was greater in the soil than in the sand. The results failed to indicate a solvent action of the decaying organic matter on rock phosphate under either leached or unleached conditions. Neither can it be concluded from these results that rock phosphate becomes more available with greater nitrification.

The effect of decaying organic matter on the availability of rock phosphate under the influence of moving capillary water

Since it was impossible to prove a solvent action of decaying organic matter on rock phosphate under leached and unleached conditions, it was thought that perhaps different results might be obtained under conditions in which the soluble phosphate would be removed as formed from the field of action by capillary water. To test out this idea six galvanized cylinders 10 inches in diameter and 2 feet high were arranged so that the water could be supplied from the bottom. A layer of quartz sand was placed on the bottom of each cylinder to a depth of 3 inches. On top of this layer was placed a layer consisting of a mixture of sand, rock phosphate and green manure. Green buckwheat and green sweet clover were used as green manure. Limestone also was added to some of the mixtures. To each mixture was added 20 cc. of filtered soil infusion. The cylinders were then filled to within 3 inches of the top with a layer of very fine pure quartz sand. Bottles were attached to maintain automatically a water level inside the cylinders below the phosphate mixtures and thus make possible a continuous capillary stream from the bottom to the top of the cylinders where the water evaporated. Small holes were provided around the cylinders at the same level as the fermenting mixtures in order to provide aeration at that point. The cylinders were kept in the greenhouse in this condition for a period of almost 8 months. At the end of this time there still remained a dry layer of about 2 inches at the surface due to evaporation of the water before it came entirely to the surface. All the dry sand was carefully removed from above the moist layer. The moist layer, to a depth of 6 inches, was then removed, thoroughly mixed and dried, and analyzed for phosphorus and calcium as follows:

Samples of 600 gm. of the dried sand were placed in flasks and boiled with 250 cc. of 1:1 HNO_3. The contents were then brought up to a volume of 750 cc. and allowed to stand until the supernatant liquid was perfectly clear. Duplicate aliquots of 250 cc. of the clear solution were taken for the analysis. The results are given in table 3.

These results show that soluble phosphorus and calcium were moved upward by the capillary water. In general, however, the presence of organic matter decreased the amount of soluble phosphorus and calcium moved upward. The slightly increased result with the combination of sweet clover and rock phosphate is within the limits of experimental error, and from the results obtained it cannot be said that the decay of organic matter has brought rock phosphate into solution.

TABLE 3

The amounts of phosphorus and calcium moved upward by capillary water from mixtures decaying organic matter and rock phosphate in fine quartz sand

TREATMENT	COMPOSITION OF DRY SOIL		POUNDS TO AN ACRE	
	P	Ca	P	Ca
	p. p. m.	*p. p. m.*	*lbs.*	*lbs.*
Rock phosphate	3.20	41.10	6.4	82.3
Buckwheat	3.15	40.60	6.3	81.2
Buckwheat, rock phosphate	3.00	32.08	6.0	64.7
Sweet clover	3.05	33.08	6.1	66.7
Sweet clover, rock phosphate	3.25	28.57	6.5	57.1
Sweet clover, rock phosphate, lime	2.95	21.05	5.9	42.1

The organic matter was used at the rate of 3 tons, rock phosphate 1 ton and limestone 2 tons to the acre.

The effect of organic matter extracts on the solubility of rock phosphate

In view of the fact that no direct solvent action could be demonstrated by composting rock phosphate in direct contact with organic matter, there remained the possibility of showing an action with the extracts of decaying organic matter. To provide the means for testing this possibility, 10 gm. each of pulverized air-dry sweet clover hay, mammoth clover hay, soybean hay, buckwheat hay, corn stover and oats straw were thoroughly mixed with 1000 gm. of pure quartz sand and placed in separate percolators. Each mixture was inoculated with 10 cc. of a filtered soil infusion and then maintained at a moisture content of 13 per cent. The mixtures were shaken vigorously at intervals and kept in a dark place in the greenhouse for a period of 6 weeks. At the end of this period each percolator was leached with 200-cc. portions of distilled water until a total of 2000 cc. of extract was secured. These extracts were filtered immediately through Pasteur-Chamberland clay filters and samples analyzed for phosphorus and calcium.

To test the solvent action of these extracts and also of distilled water on rock phosphate, a battery of percolators was arranged in two sets; so that tests could be made with and without the addition of carbon dioxide. The carbon dioxide was used in one set to make the conditions more comparable to that of the field. To each percolator was added 600 cc. of the extract and 4.6 gm. of rock phosphate, which is the proportion of rock phosphate and water

used in the decomposition experiments and in pot cultures carried on in this investigation. Effective agitation was maintained in the one case by the bubbling of CO_2 from a tank, and in the other by the aspiration of CO_2-free air. After 48 hours of such contact at room temperature the extracts were siphoned off and filtered through Pasteur-Chamberland clay filters. The extracts were analyzed for phosphorus and calcium as before. Where the CO_2 was used there was an appreciable disappearance of color in all solutions, indicating the precipitation of the soluble and colloidal organic matter in the solutions. The results of these studies are given in table 4.

Of the original extracts, the buckwheat extract contained the largest amount of soluble phosphorus and calcium, while the corn stover and oats-straw extracts contained the smallest amounts. In the absence of carbon dioxide, agitation of the extracts with rock phosphate decreased the amounts of soluble phosphorus and calcium, save in two cases where slight increases of soluble phosphorus occurred which were within the limits of experimental error. In the presence of carbon dioxide agitation of the extracts with rock phosphate gave no regular or definite increases of soluble calcium, but the soluble phosphorus increased in all cases except with the buckwheat extract. The distilled water alone dissolved an appreciable amount of phosphorus and calcium, and with carbon dioxide it dissolved more phosphorus and calcium than were found in solution in the extracts treated similarly. The presence of calcium carbonate as an impurity in the commerical rock phosphate may be a factor which causes the soluble phosphorus to go out of solution in fermenting mixtures of organic matter and rock phosphate. Undoubtedly, carbon dioxide changes some of this into calcium bicarbonate which may react with soluble phosphates and precipitate them. The contact of soluble tricalcium phosphate with soluble mono-calcium phosphate tends toward an equilibrium in which some of the soluble phosphate reverts to the more insoluble di-calcium

TABLE 4

The amounts of phosphorus and calcium in organic matter extracts before and after contact with rock phosphate and with and without carbon dioxide

KIND OF EXTRACT OR LIQUID	ORIGINAL CONTENT		CONTENT AFTER CONTACT WITH ROCK PHOSPHATE			
			Without CO_2		With CO_2	
	P	Ca	P	Ca	P	Ca
	p. p. m.	*p. p. m.*	*p. p. m.*	*p. p. m.*	*p. p. m.*	*p. p. m.*
Distilled water			1.92	9.62	5.84	28.07
Buckwheat hay extract	7.10	39.09	4.80	14.44	5.20	26.47
Soybean hay extract	4.30	31.08	2.72	14.44	4.72	26.66
Sweet clover hay extract	4.05	22.05	3.12	13.23	4.84	24.06
Mammoth clover hay extract	3.45	22.56	3.76	11.63	4.72	19.25
Corn stover extract	3.44	16.04	3.48	13.23	4.96	21.85
Oats straw extract	3.00	17.24	2.88	16.04	4.24	24.06

phosphate. DeTurk (8) in a somewhat similar manner found that the amount of soluble potash decreased in fermenting organic mixtures containing insoluble potash minerals. He explained his results as due to the direct absorption of the soluble potash by the minerals.

In considering the reasons for the decrease of the soluble phosphorus in fermenting mixtures, the use of soluble phosphorus by the bacteria, as pointed out by Tottingham and Hoffman (41) must not be overlooked. In the experiments with the organic liquids, however, it is not likely that bacteria had much influence. No changes due to nitrification could be detected in these extracts.

The relation of decaying organic matter and rock phosphate to plant growth

In view of the fact that chemical studies made with decaying organic matter did not show any solvent action on rock phosphate, the question still remained as to the effect of such mixtures on the growth of plants. This problem was investigated with a series of sand cultures in which corn was grown with several forms of organic matter in various combinations with rock phosphate and limestone, as indicated in table 5.

As sources of organic matter, finely pulverized alfalfa hay, buckwheat hay, oats straw and chopped green buckwheat hay were used. The dry materials contained 0.212, 0.282 and 0.113 per cent of phosphorus, respectively. The green buckwheat was not analyzed. Each kind of organic matter was used in two amounts. The larger application of each contained approximately 50 mgm. of phosphorus which was sufficient for a good growth of corn provided it could all be used. The smaller application was approximately one-third of the larger, which was inadequate for good growth, yet furnished sufficient phosphorus to give the plant a good start and enough organic matter to have a solvent effect on the rock phosphate. Checks were maintained without organic matter and without phosphorus. The nutrient solution used was the same as that employed in the sand cultures previously reported (3) in which $NaNO_3$ was the form of nitrogen salt added, except that half of the $NaNO_3$ was replaced with an equivalent amount of $Ca(NO_3)_2$. The cultures were managed in a manner similar to the method employed with previous ones with respect to moisture and other conditions. All cultures were duplicated.

Shortly after germination the corn showed evidences of toxic effects where the organic matter, with the exception of the green buckwheat, had been used. With the alfalfa and the dry buckwheat these effects disappeared after several weeks. Where the limestone was used the toxic effects were much less. The cultures with oats straw, however, never recovered and most of the plants finally perished. Because of lack of time, the plants were harvested after 60 days of growth. A longer period of growth would undoubtedly have shown more striking results. The yields of dry matter are given in table 5.

The results show that the phosphorus in the organic matter was readily available to growing corn. The dry buckwheat was by far the most efficient. When the application of buckwheat hay was reduced to one-third, the growth was also reduced to about one-third. The difference in the case of the alfalfa hay due to the amount used was not so marked. The largest growth of all occurred where the large quantity of dry buckwheat was applied.

All combinations of organic matter and rock phosphate gave substantial increases in the yield of dry matter over that produced by either the organic matter or rock phosphate when used alone. Increases from the combination

TABLE 5

The amounts of dry matter produced in quartz sand corn cultures with mixtures of organic matter, rock phosphate and limestone

FORM OF ORGANIC MATTER	AMOUNT OF ORGANIC MATTER USED	SUPPLEMENTARY TREATMENTS*	DRY MATTER PRODUCED			INCREASE OF TOTAL DRY MATTER OVER CHECK	INCREASE OF TOTAL DRY MATTER WITH MIXTURE OF ROCK PHOSPHATE AND ORGANIC MATTER OVER COMBINED SEPARATE INCREASES DUE TO THESE
			Tops	Roots	Total		
	gm.		gm.	gm.	gm.	gm.	gm.
None (check).	0	None	2.6	1.6	4.2		
	0	Rock phosphate	5.5	3.8	9.3	5.1	
Dry alfalfa hay........	25	None	4.1	3.1	7.2	3.0	
	8	None	2.4	1.8	4.2	0.0	
	8	Rock phosphate	6.8	3.6	10.4	6.2	1.1
	8	Rock phosphate, lime	9.4	4.2	13.6	9.4	4.3
Dry buck-wheat hay..	20	None	16.0	5.1	21.1	16.9	
	6	None	4.6	2.2	6.8	2.6	
	6	Rock phosphate	11.2	5.5	16.7	12.5	4.8
	6	Rock phosphate, lime	7.6	4.3	11.9	7.7	0.0
Green buck-wheat tissues	50	None	5.8	4.2	10.0	5.8	
	50	Rock phosphate	8.6	6.2	14.8	10.6	−0.3

* Rock phosphate used at the rate of 1 ton and limestone at the rate of 2 tons to the acre.

of organic matter and phosphate over the combined increases from these materials used separately are not so marked. Indeed, the combination of green buckwheat and rock phosphate resulted in a slight decrease of dry matter as compared with the combined separate increases of these materials. In the case of alfalfa there was a slight increase, and where limestone was used in addition, there was quite a marked increase. The combination of dry buckwheat and rock phosphate gave the largest increase of dry matter of all cultures over the combined separate increases of these materials, but where limestone was used in addition, there was no increase at all when calculated on this same basis.

The increases from the combination of organic matter and rock phosphate may have been due to one or more reasons. The organic matter furnished available phosphorus to the seedling and thereby promoted growth which may have enabled the plant to feed more strongly on the rock phosphate, or the rock phosphate may have been made more available by the chemical and biological processes accompanying the decay of the organic matter. However the fact that green buckwheat did not produce toxic effects and that the combination of green buckwheat and rock phosphate produced no increase over the combined separate increases of these materials, makes it questionable whether or not the organic matter increased the availability of the rock phosphate in any of the cases. The combination of alfalfa and rock phosphate which was toxic in the early stages of growth produced a marked increase only when limestone also was added, a fact which indicated that the lime prevented the toxic effect of the organic matter. The dry buckwheat combination, which did not appear to be quite as toxic as the alfalfa combination, produced a large increase for the combination, but none when the lime was used in addition. It thus appears that the rock phosphate itself may have lessened the toxic effects produced by the organic matter. Although there is some evidence that the combination of organic matter and rock phosphate increased the availability of the latter to corn, the complication produced by the toxic effects of the organic matter does not permit definite conclusions in this respect.

TABLE 6

The total and water-soluble phosphorus and calcium in plant tissues

KIND OF TISSUE	TOTAL		WATER-SOLUBLE		PERCENTAGE OF TOTAL WATER-SOLUBLE		CONTENTS OF 1 TON, WATER-SOLUBLE		
	P	Ca	P	Ca	P	Ca	P	Ca	Phosphorus equivalent to that in corn crop of
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*lbs.*	*lbs.*	*bu.*
Buckwheat hay	0.318	1.544	0.262	0.080	82.1	5.2	5.24	1.6	22.5
Sweet clover hay	0.240	1.423	0.218	0.672	90.8	47.1	4.36	13.44	19.0
Mammoth clover hay	0.244	1.644	0.216	0.681	88.5	41.4	4.32	13.62	18.7
Corn stover	0.290	0.642	0.198	0.190	68.2	29.6	3.96	3.80	17.2
Oats straw	0.230	0.621	0.142	0.220	61.7	35.4	2.82	4.40	12.3

In this connection the amount of water-soluble phosphorus in some of the common farm residues is of interest. In table 6 are given the amounts of total and water-soluble phosphorus and calcium in five agricultural crops. For the water-soluble phosphorus and calcium determinations, 5-gm. samples of pulverized material were shaken vigorously by hand with 500 cc. of distilled water for about 5 minutes and allowed to settle, after which the supernatant liquid was filtered in the usual way. Aliquot portions of 100 cc. were

taken for analysis. The organic matter present was oxidized with bromine water, after which the phosphorus and calcium were separated by the basic acetate method and determined in the usual manner.

The results show that 90.8 per cent of the total phosphorus in the sweet clover hay was water-soluble, while only 61.7 per cent of the total phosphorus of oats straw was water-soluble. Of the total calcium in sweet clover and buckwheat hay 47.1 per cent in the former and only 5.2 per cent in the latter was water-soluble. The soluble phosphorus in a ton of sweet clover hay is equal to the total phosphorus in 19.0 bushels of corn and correspondings stalks, while that in a ton of oats straw is equal to that in only 12.3 bushels of corn and corresponding stalks. The data of the Ohio Agricultural Experiment Station (1) indicate that alfalfa contains about the same proportion of water-soluble phosphorus as is herein reported for sweet-clover hay.

The ability of plants to feed directly on rock phosphate

In view of the fact that the return of crop residues to the soil furnishes available phosphorus to succeeding crops which may feed poorly on rock phosphate, the value of having some crops in a rotation which are able to feed strongly on rock phosphate, assumes considerable importance. It seems that the use of such crops on phosphorus-deficient soils should make possible a more effective use of rock phosphate. The value, therefore, of knowing more about the ability of various plants to feed directly on rock phosphate becomes apparent.

In most samples of rock phosphate there is a small but appreciable portion of phosphate which is readily soluble, perhaps by hydrolysis. Undoubtedly this small portion of readily soluble and available phosphate in rock phosphate is an important factor in studying the feeding power of plants for rock phosphate. This study therefore may be made in two ways. First, the rock phosphate may be used in small amounts so that the total phosphorus applied, if all available, would be just slightly more than that necessary for good plant growth. When such small amounts are used, the amount of readily available phosphate will be practically negligible as far as this study is concerned and hence the growth made by a plant will depend on its true feeding power for the kind of phosphate which makes up the greater portion of the rock phosphate. In the second place, the rock phosphate may be used in larger amounts, such as that commonly advised in field practice. With such applications much more total phosphorus is applied than is necessary for good plant growth. Obviously the amount of readily available phosphorus is thus also proportionately increased. When plants of low feeding power but possessing rather extensive and fibrous roots systems are grown under these conditions, growth will be proportionately greater than that of plants of similar feeding power, but possessing less extensive root systems, because of the ability of the former to utilize the phosphorus made available by hydrolysis. This method will

TABLE 7

The yields of dry matter under the treatments indicated and the proportion of roots and percentage normal growth of plants grown with rock phosphate

CROP	PART OF PLANT	TREATMENT OF PHOSPHATE AND LIME: None	Acid phosphate	Rock phosphate	Rock phosphate, lime	PROPORTION OF ROOTS TO WHOLE PLANT WHEN ROCK PHOSPHATE WAS USED	PERCENTAGE NORMAL GROWTH ON ROCK PHOSPHATE COMPARED WITH ACID PHOSPHATE AT 100
		gm.	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*
Red clover	Tops	0.1	16.4	4.1	3.0	43.1	33.3
	Roots	0.4	5.2	3.1	2.9		
	Total	0.5	21.6	7.2	5.9		
Wheat	Tops	0.6	25.3	7.7	3.3	42.1	34.4
	Roots	1.3	13.4	5.6	3.0		
	Total	1.9	38.7	13.3	6.3		
Oats	Tops	1.0	42.0	20.1	18.8	15.9	41.5
	Roots	1.8	15.7	4.6	3.6		
	Grain	0.0	12.2	4.3	3.9		
	Total	2.8	69.9	29.0	26.3		
Corn	Tops	1.9	45.2	18.3		25.3	41.9
	Roots	1.2	13.3	6.2			
	Total	3.1	58.5	24.5			
Timothy	Tops	0.3	12.3	5.3	5.6	30.3	45.2
	Roots	0.7	4.5	2.3	3.0		
	Total	1.0	16.8	7.6	8.6		
Soybeans	Tops	3.6	17.2	7.3	6.4	28.1	47.7
	Roots	2.3	4.4	2.9	3.0		
	Total	5.9	21.6	10.3	9.4		
Rape	Tops	1.0	17.8	8.6	9.5	26.5	54.1
	Roots	0.5	3.8	3.1	5.7		
	Total	1.5	21.6	11.7	15.2		
Alfalfa	Tops	0.1	9.8	5.2	4.5	50.9	62.3
	Roots	0.2	7.2	5.4	4.6		
	Total	0.3	17.0	10.6	9.1		
Rye	Tops	0.6	15.6	7.2	6.8	64.9	66.9
	Roots	1.3	15.0	13.3	14.5		
	Total	1.9	30.6	20.5	21.3		
Buckwheat	Tops	1.3	14.9	10.1	4.8	17.2	72.1
	Roots	0.5	2.0	2.1	1.2		
	Total	1.8	16.9	12.2	6.0		

TABLE 7—*Continued*

CROP	PART OF PLANT	TREATMENT OF PHOSPHATE AND LIME: None	Acid phosphate	Rock phosphate	Rock phosphate, lime	PROPORTION OF ROOTS TO WHOLE PLANT WHEN ROCK PHOSPHATE WAS USED	PERCENTAGE NORMAL GROWTH ON ROCK PHOSPHATE COMPARED WITH ACID PHOSPHATE AT 100
		gm.	*gm.*	*gm.*	*gm*	*per cent*	*per cent*
Redtop	Tops	0.3	21.8	12.0	10.8	49.6	72.3
	Roots	0.7	11.1	11.8	10.3		
	Total	1.0	32.9	23.8	21.1		
Red sorrel	Tops	0.3	13.3	8.7	5.5	59.4	82.9
	Roots	0.4	12.5	12.7	9.8		
	Total	0.7	25.8	21.4	15.3		
Sweet clover	Tops	0.3	15.2	12.7	14.3	35.2	83.0
	Roots	0.8	8.4	6.9	6.4		
	Total	1.1	23.6	19.6	20.7		
Mammoth clover	Tops	0.3	10.5	5.0	2.4		
Alsike clover	Tops	0.1	6.6	3.8	2.1		

give information as to the relative order of plant growth under these conditions rather than the true feeding power of the plant. It is the method employed in the investigations reported herein.

To test the relative order of growth of various crops on rock phosphate, fifteen different kinds of plants were grown in sand cultures with rock phosphate with and without limestone. For comparison, plants were grown also without phosphorus and with acid phosphate.

The average yields of the dry matter of the tops, roots and total plants, the proportion of roots to the whole plant when grown with rock phosphate and the percentage growth made with rock phosphate compared with that made with acid phosphate, are recorded in table 7.

The results show that there was quite a wide variation in the growth of the different plants with rock phosphate used at the rate commonly advised in field practice. All the plants possessed some power to utilize the phosphorus of rock phosphate, as was indicated by the lesser growth on the check pots. Sweet clover and red sorrel produced the largest proportionate increases of dry matter and the red clover the smallest. The increases of the former were about two and one-half times as much as the latter. The use of limestone usually decreased plant growth, because of a lower availability of the phosphorus, though in a few cases it caused a slight increase in the amount of dry matter produced.

As a general rule the root systems became proportionately greater when rock phosphate was substituted for acid phosphate and still greater when limestone was used with the rock phosphate. This was evidently due to the

greater activity of the roots in trying to secure phosphorus which became increasingly more unavailable. Sweet clover, however, was an exception to this general rule. The proportion of roots to the whole plant was not definitely related to the amount of plant growth made with rock phosphate. Buckwheat and sweet clover made relatively large growths and had comparatively small root systems. Red sorrel and rye grew well and had relatively large proportions of roots. On the other hand, wheat and red clover grew poorly and had relatively high proportions of roots.

In table 8 are given the phosphorus and calcium contents of the tops and the acidity values for the leaves, stems and roots of some of the plants grown with both acid and rock phosphate.

TABLE 8

The phosphorus and calcium content of plant tops and acidity of the leaves, stems and roots of plants grown with acid and rock phosphates

CROP	PHOSPHORUS CONTENT OF PLANT GROWN WITH		CALCIUM CONTENT OF PLANT GROWN WITH		ACIDITY OF PLANT JUICES					
					Grown with acid phosphate			Grown with rock phosphate		
	Acid phosphate	Rock phosphate	Acid phosphate	Rock phosphate	Leaves	Stems	Roots	Leaves	Stems	Roots
	per cent	*per cent*	*per cent*	*per cent*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
Alfalfa	0.283	0.177	1.15	0.96	5.14	5.14	5.39	8.15	8.35	7.81
Buckwheat					5.65	4.46	4.72	5.65	3.96	4.89
Corn	0.135	0.083	0.57	0.56	5.36	5.36	6.04	5.36	5.67	6.02
Rape	0.372	0.222	1.59	1.00	5.56	5.70	5.48	5.38	5.56	5.34
Red clover	0.283	0.153	1.01	0.93	6.14	5.82	6.83	4.63	3.67	4.89
Redtop	0.301	0.206	0.63	0.42						
Rye					6.07	7.10	6.65	6.58	6.53	7.59
Red sorrel	0.403	0.311	0.94	0.59	2.93	3.63	5.82	2.79	2.99	5.51
Soybean	0.434	0.208	1.38	0.77	5.82	5.50	6.33	5.99	6.21	6.58
Sweet clover	0.310	0.314	1.37	1.36	5.75	5.82	5.97	6.49	6.53	7.17
Timothy	0.359	0.280	0.36	0.24						
Wheat					4.52	4.63	5.31	4.38	4.89	4.68

With the exception of sweet clover which gave a slight increase, all the plants analyzed gave rather large decreases in the percentage content of phosphorus when grown on rock phosphate as compared with acid phosphate. In general, neither the percentage content, nor the total amount of phosphorus in the plant tops was related to the amount of plant growth made with rock phosphate.

The calcium content of the top portions of the plants was always greater in the plants grown on acid phosphate than in those grown on rock phosphate, though in a few cases the difference was very slight. If the relation of the calcium content of the plants to the growth produced with large applications of rock phosphate is compared with the relation of the calcium content to the true feeding power of the plant for rock phosphate as enunciated by Truog

(44), it is seen that the two relations do not agree in all cases. Plants with extensive and fibrous root systems and low calcium content such as timothy and red top, produced more growth with large applications of rock phosphate than plants with somewhat higher calcium contents but less extensive root systems. This was due undoubtedly to the greater ease with which such plants were able to get the readily available portion of phosphorus from the large amounts of rock phosphate used. Red clover with a high calcium content grew but poorly on rock phosphate. In this respect the behavior of red clover, even with the larger application of rock phosphate, is similar to that obtained by Kossowitch (21) and Truog (44). In studying the feeding power of plants for rock phosphate, Truog attributed the poor showing of red clover to the extremely small seeds which were unable to furnish the seedling with sufficient phosphorus for a good start. He was able to demonstrate that alfalfa and tobacco, both small-seeded plants of high calcium content, became much better feeders after the root systems were more thoroughly developed. Of the cultures reported herein, sweet clover, mammoth clover, and alsike clover, all small-seeded plants of high calcium content, grew much better on rock phosphate than red clover, a fact which suggests the possibility of other undertermined factors. It was observed, however, that of all the plants grown, red clover produced the largest increase in the proportion of roots when rock phosphate replaced acid phosphate, and also the largest increase when limestone was used with the rock phosphate. The acidity of the juices of red clover grown on rock phosphate was one of the highest from all of the plants tested. The acidity of the juice of red clover grown on acid phosphate was slight.

The acidity values of the juice of the leaves, stems and roots varied considerably with the different plants. In general the juices of the plants were more acid when grown with acid phosphate than with rock phosphate. Wheat and red clover, however, were more acid when grown with rock phosphate, a fact which may be related to the poor growth of these plants with rock phosphate. On the whole, there was no relation of the acidity of the plants to their ability to feed directly on rock phosphate. Red sorrel and sweet clover both grew well on rock phosphate; the former was high in acidity, while the latter was low.

In connection with the theory that plants high in calcium are good feeders on rock phosphate, it was thought that the testing of the feeding powers of a few typical plants for the potash in feldspar, in which case the removal of calcium is not a factor, would be of interest. For this test, corn, oats, sweet clover and buckwheat were grown in sand cultures with rock phosphate. Finely powdered feldspar at the rate of 2 tons to the acre was used as the source of potassium. The nutrient solution was modified from that used in the phosphate studies by substituting mono-calcium phosphate for the potassium sulfate. Checks without potash and with potassium sulfate were used for comparison. The sand used was previously leached to remove any soluble

potassium. The work was done in duplicate. The growth of the plants with the soluble potash was not so good as desired. Of the four crops, sweet clover grew the nearest to normal. The reason for the lack of vigor of the other crops is not known. This condition made the feeding powers of the plants on feldspar appear abnormally high. The crops were harvested 50 to 60 days from the time of seeding, and the results are recorded in table 9.

The oats and sweet clover grew as well on the feldspar as on the soluble potash. Corn and buckwheat, however, did very poorly on the feldspar. The chief differences between the feeding power of these plants for feldspar and rock phosphate is in the position of oats and buckwheat. With rock phosphate buckwheat produced a large growth; with feldspar it scarcely

TABLE 9

The growth of plants in quartz cultures with no potash, soluble potash and feldspar potash

KIND OF PLANT	PART OF PLANT	YIELDS OF DRY MATTER WITH TREATMENTS AS FOLLOWS			NORMAL GROWTH ON FELDSPAR
		None	Soluble potash	Feldspar	
		gm.	*gm.*	*gm.*	*per cent*
Buckwheat	Tops	1.0	7.4	1.6	
	Roots	0.5	2.0	0.6	
	Total	1.5	9.4	2.2	23.3
Corn	Tops	5.0	26.5	9.5	
	Roots	2.5	4.5	4.5	
	Total	7.5	31.0	14.0	45.2
Sweet clover	Tops	2.5	5.4	5.4	
	Roots	1.0	2.2	2.7	
	Total	3.5	7.6	8.1	106.5
Oats	Tops	5.0	8.3	7.7	
	Roots	1.2	2.2	3.6	
	Total	6.2	10.5	11.3	107.6

grew. Oats showed the opposite condition, though not to such a marked extent. The only explanation that can be given for this reversal in the growth of these plants is the difference in the character of the root systems of the two crops. Buckwheat has a scanty root system. Oats has an extensive and fibrous root system, making it possible for the oats to absorb the soluble potassium produced by hydrolysis or otherwise, much more completely than buckwheat. Although the results with feldspar are somewhat irregular, an important point is indicated. Buckwheat feeds poorly on feldspar, but strongly on rock phosphate. The reason for this reversal is found in the theory that plants high in calcium are strong feeders on rock phosphate. In the case of feldspar there is no soluble calcium to remove, and the ability to utilize the soluble potassium is probably determined largely by the extent

of the root system. The oat plant has an extensive root system and thus feeds strongly on it.

Of all the plants grown in sand cultures with rock phosphate and feldspar sweet clover showed the most remarkable feeding power. To demonstrate further the feeding power of sweet clover for relatively insoluble plant-food materials, inoculated seeds were planted in pure quartz sand in 2-gallon pots. Rock phosphate was added at the rate of 1 ton, and dolomitic limestone and feldspar each at the rate of 2 tons to the acre. Distilled water was used to water the plants. The first application of water contained the regular amount of $NaNO_3$ used in the pots-culture studies previously reported. In the cousre of time a fairly luxuriant growth of sweet clover resulted. A similar experiment with corn resulted in only a very small growth with the production of tassels when the plants were about 12 inches high.

Just why sweet clover is such a good feeder is not entirely clear. The fact that it is high in calcium aids in the explanation of its feeding powers for rock phosphate. However, this explanation will not hold for its high feeding power for feldspar. There are evidently other factors which are important in this connection.

In addition to its strong feeding powers, sweet clover is a crop which lends itself well to rotation schemes. It may be seeded in small grain and used for pasture and hay purposes the next season, or plowed down in the preparation of corn land the following spring. The last method is especially well adapted to grain systems of farming for the effective use of rock phosphate. Undoubtedly there are many ways in which the farmer could advantageously use the strong feeding powers of plants like sweet clover, rape and buckwheat.

SUMMARY

It is commonly believed that organic acids, carbon dioxide and nitrous acid resulting from the decay of organic matter are active agents in making rock phosphate available to plants. When the effect of organic matter on the availability of rock phosphate has been studied in the laboratory, increases of water- or citrate-soluble phosphorus have rarely been found. These laboratory experiments have usually been made without provision for the removal of the phosphorus and calcium as made soluble, as is the case under field conditions. For this reason it seemed highly desirable to conduct further studies on this problem. Experiments were planned in which common forms of organic matter were used with rock phosphate. In some of these experiments provision was made for the removal of the soluble phosphorus and calcium in order more nearly to imitate field conditions in this respect.

Experiments were made as follows: (a) rock phosphate was mixed with several forms of organic matter in sand and soil with no provision for the removal of phosphorus as it became soluble; (b) in similar mixtures provision was made by leaching for the removal of phosphorus as it became soluble;

(c) in still other similar mixtures provision was made by upward-moving capillary water for the removal of phosphorus as it became soluble; (d) in a fourth experiment the solvent effect of organic-matter extracts with and without carbon dioxide on rock phosphate was studied; (e) pot culture experiments also were made in this problem, and in addition the feeding powers of plants in relation to the utilization of rock phosphate and feldspar were investigated. The results are summarized as follows:

1. Experiments (a), (b), (c) and (d) failed to show a solvent effect of the decaying organic matter on rock phosphate; and in accord with the results of several other investigators the results in all cases showed a decreased amount of soluble phosphorus from that present at the beginning.

2. The failure of the experiments to show an increase in the availability of rock phosphate was probably due to the fact that when organic matter such as was used, decomposes, sufficient bases are liberated along with the acids that are formed, to neutralize the acids and prevent their action on rock phosphate. Some of the soluble phosphorus possibly was used by bacteria, as indicated by Tottingham and Hoffman (41). When mixtures of rock phosphate and organic matter are applied to soils, the conditions are different, because of the capacity of soils to take up basic material, especially if they are acid.

3. In some cases mixtures of organic matter and rock phosphate applied to pot cultures produced increases in the growth of corn over the combined separate increases of organic matter and rock phosphate. The phosphorus of the organic matter became available readily to corn. The decaying organic matter either had a solvent action on the rock phosphate, or else the phosphorus supplied by the organic matter gave the corn seedlings a better start, so that they were enabled to exert a stronger feeding power toward the rock phosphate.

4. A study of the growth of fifteen different plants on rock phosphate in sand cultures showed a wide variation in the amount of dry matter produced. There was no definite relation of the amount of plant growth to the extent of root systems, the phosphorus content, or the acidity of the plant juices. The applications of rock phosphate were too large to allow a critical examination of the relation between the feeding power and the calcium content.

5. The greater ability of some plants which are high in calcium to feed on rock phosphate than on feldspar is in accord with the theory that plants high in calcium are good feeders on rock phosphate.

6. Sweet clover possesses remarkable feeding powers toward rock phosphate and feldspar and is well suited to rotations for the utilization of these.

7. The possibility of growing crops of high feeding power to supply organic matter and available phosphorus in rotations with crops of low feeding power is a question of considerable practical importance in the utilization of rock phosphate.

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EXPERIMENTS IN THE TREATMENT OF BALLED EARTH ABOUT THE ROOTS OF CONIFEROUS PLANTS FOR THE CONTROL OF JAPANESE BEETLE LARVAE

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INTRODUCTION

A Federal plant quarantine in operation during the spring of 1920 prohibited the interstate shipment of soil or coniferous plants with soil about their roots from within a restricted area in New Jersey infected by the Japanese beetle (*Popillia japonica*). As a result of this quarantine two nurseries engaged in growing coniferous and certain other ornamental stock such as azaleas, were restricted in the marketing of their product, a condition rendered necessary by the fact that no safe method exists for the killing of insects which may be contained within the soil about the roots of plants. Under these circumstances a series of experiments were conducted during 1920 at Riverton, N. J. to determine the comparative value of a certain class of *gas-producing* compounds as killing agents against the soil-inhabiting Japanese beetle grub and also the effect of these toxic compounds upon the plants concerned.

NURSERY PRACTICE IN SHIPPING CONIFEROUS PLANTS

In order that coniferous plants, such as Arbor vitae, spruces, pines, etc., may be successfully transplanted, it is essential that the tree be dug in such a way that a sufficient proportion of the roots and rootlets are not disarranged in the soil or exposed to the drying action of the atmosphere. Figure 1 of plate 1 illustrates a small conifer which has been carefully dug from the nursery row so as to preserve the roots within the soil-ball. In order to secure a good soil-ball about the roots nurserymen transplant conifers at frequent intervals in the nursery row. This frequent transplanting with the accompanying root pruning, results in a compact mass of fibrous roots in the immediate vicinity of the base of the tree. This compact mass of roots and soil, or or the *soil-ball* as it is called, facilitates handling and insures successful transplanting. As an added precaution in shipping this class of stock the soil-ball is wrapped in burlap (pl. 1, fig. 2).

POSSIBILITY OF INFESTATION OF SOIL-BALLS BY INSECTS; THE LONG LARVAL PERIOD OF THE JAPANESE BEETLE

The possibility of these soil-balls being infested with soil-inhabiting animals such as root aphids, wire-worms, white grubs, nematodes, etc., has been confirmed by the finding of various specimens in the loose soil in the bottom and sides of the holes from which balled plants had been removed. An examination of a block of azaleas in one of the local nurseries disclosed the presence of larvae of the Japanese beetle in among the plants at one end of the block, the infestation no doubt resulting from the fact that the rows at that point were somewhat weedy, a condition favorable to the deposition of eggs by the Japanese beetle. In this connection it is well to remember that the larvae of *Popillia japonica* are found in the soil throughout the year, young grubs just hatched from the eggs being present in the soil before all the old grubs have pupated and emerged as beetles. There therefore exists no fixed period in the year within which balled plants could be shipped with the absolute assurance that no grubs would be contained within the shipment.

BASIS OF THE EXPERIMENTAL WORK

As stated above, this class of nursery stock when ready for shipment is tightly wrapped in burlap bagging. Under these circumstances the plants can be dipped or soaked in solutions with impunity as far as mechanical injury is concerned, since only a very small proportion of the soil escapes through the bagging and the relative position of the roots within the soil-ball is undisturbed. On removal from the solution the plants would be ready for shipment after being allowed to drain for a few hours.

CLASSES OF COMPOUNDS EMPLOYED

The following classes of compounds were employed dissolved in water.

I. Gas-producing compounds soluble in water.
   a. Such compounds slightly soluble in water, e.g., carbon disulfide.
   b. Such compounds readily soluble in water, e.g., sodium sulfocarbonate.
II. Corrosive compounds, e.g., copper sulfate.

EXPERIMENTAL PROCEDURE

Aside from the few initial experiments the following steps were taken in testing the various compounds:

I. *Popillia* grubs were dipped in solutions of varying concentrations of the material being tested for varying periods of time. Throughout this set of dipping tests the solutions were maintained at a temperature of approximately 70°F.

II. If a compound gave evidence of positive value as a killing agent when used as above a second series of experiments were made with the material.

In these latter experiments the grubs, *embedded in soil-balls*, were subjected to the action of the material in solution.

III. The material in solution was tested as to its action on the plant, small coniferous plants with balled earth about their roots being employed.

DIPPING TESTS WITH GRUBS OF POPILLIA JAPONICA

In making these tests 1,000 cc. of the solution was placed in a glass beaker. The grubs were prevented from sinking to the bottom of the beaker by means of a muslin or wire screen, the object being to retain the grubs in the exact vertical center of the solution. The results of these dipping tests are given in tables 1, 2, and 3.

Sodium sulfocarbonate

Preparation. This material was prepared from powdered fused sodium sulfide and carbon disulfide by agitation. The sodium monosulfide (Na_2S) deliquescent crystals were melted, the liquid poured into a shallow pan, allowed to harden and ground to a powder in a mortar. A quantity of this powder was then placed in a mason jar with twice the volume of carbon disulfide and agitated at short intervals for 2 hours. If larger quantities are desired a kitchen butter-churn lends itself to this purpose. The resulting sodium sulfocarbonate is heavier than either the excess carbon disulfide or sludge of impurities remaining after the chemical action is complete. On standing for a short time the excess carbon disulfide rose to the top of the mixture and was decanted. The remaining mixture of sodium sulfocarbonate and sludge was then filtered, the sludge remained on the filter-paper while the filtrate consisted of sodium sulfocarbonate.

Carbon disulfide acts as an acid toward alkaline sulfides. The reaction with sodium sulfide is as follows:

$$Na_2S + CS_2 = Na_2CS_3$$

Properties. The sodium sulfocarbonate resulting from the above method of preparation is a heavy, reddish liquid and tests about 45° Baumé. It is soluble in water in all proportions. The compound is unstable and reacts with water and carbon dioxide in the air or soil. On decomposing, it yields about 20 per cent carbon disulfide and 9 per cent hydrogen sulfide (1). The reaction is as follows:

$$Na_2CS_3 + CO_2 + H_2O = Na_2CO_3 + CS_2 + H_2S.$$

Organic acids produce the same result. The reaction with acetic acid is as follows:

$$Na_2CS_3 + 2HC_2H_3O_2 = 2NaC_2H_3O_2 + CS_2 + H_2S.$$

Table 1 gives the results of dipping *Popillia* grubs in various solutions of sodium sulfocarbonate, the grubs *not* embedded in soil. When grubs were

dipped in a solution of 15 cc. of sodium sulfocarbonate per gallon for 220 minutes, 100 per cent mortality resulted. On the other hand, when grubs were dipped in a solution of 15 cc. of sodium sulfocarbonate and 15 cc. of acetic acid per gallon the grubs were all dead in 30 minutes. These results indicate that while sodium sulfocarbonate itself has some killing ability the active killing agent is the carbon disulfide resulting from the decomposition of the sodium sulfocarbonate by organic acids.

TABLE 1

Results of dipping Popillia grubs in solutions of sodium sulfocarbonate; grubs in the third (last instar and not in soil*

CONCENTRATION OF SOLUTION	DURATION OF DIPPING	Kill
	min.	*per cent*
30 cc. sodium sulfocarbonate per gallon	30	33
30 cc sodium sulfocarbonate per gallon	60	33
30 cc. sodium sulfocarbonate per gallon	90	66
30 cc. sodium sulfocarbonate per gallon	120–230	100
15 cc. sodium sulfocarbonate per gallon	30	0
15 cc. sodium sulfocarbonate per gallon	60	33
15 cc. sodium sulfocarbonate per gallon	120	66
15 cc. sodium sulfocarbonate per gallon	220–270	100
15 cc. sodium sulfocarbonate plus 15 cc. 36 per cent acetic acid per gallon	15	66
15 cc. sodium sulfocarbonate plus 15 cc. 36 per cent acetic acid per gallon	30–120	100
30 cc. sodium sulfocarbonate plus 15 cc. 36 per cent acetic acid per gallon	15	0
30 cc. sodium sulfocarbonate plus 15 cc. 36 per cent acetic acid per gallon	30	33
30 cc. sodium sulfocarbonate plus 15 cc. 36 per cent acetic acid per gallon	60–120	100
30 cc. sodium sulfocarbonate plus 30 cc. 36 per cent acetic acid per gallon	15	100
30 cc. sodium sulfocarbonate plus 30 cc. 36 per cent acetic acid per gallon	30–120	100
Water only (checks)	15–300	0

* Tests were made using first and second instar grubs and pupae of *Popillia japonica*. The tests indicate that the first and second instar grubs are only slightly less resistant to toxic compounds than third instar grubs while the pupae are equal to the third instar grub in resistance.

Sodium ethyl xanthate

Preparation and properties. Sodium ethyl xanthate[1] was made as follows: 200 gm. of sodium hydroxide was dissolved in 200 cc. of water and the mixture cooled. To this was added 300 cc. of alcohol and the mixture shaken. Then 310 cc. of carbon disulfide was added in small portions, with shaking and cooling after each addition to prevent loss of CS_2

[1] The writers are indebted to Dr. C. C. McDonnell, Insecticide and Fungicide Board, U. S. D. A., for the preparation of this material.

which would occur if the flask were allowed to heat above approximately 45°. A slight excess of carbon disulfide and alcohol was used in order to get as nearly as possible to a neutral reaction. The product obtained should contain about 70 per cent of the crude xanthate. This product is of course not as pure as that made under anhydrous conditions.

The reactions are as follows:

$$H_2O + NaOH + C_2H_5OH = NaOC_2H_5 + H_2O.$$
$$NaOC_2H_5 + H_2O + CS_2 = C_2H_5OCSSNa + H_2O.$$

Sodium ethyl xanthate is orange yellow in color and is readily soluble in water. Decomposition of the aqueous solution into carbon disulfide, ethyl

TABLE 2

Results of dipping Popillia grubs in solutions of sodium ethyl xanthate in water; grubs in the third (last) instar, and not in soil

CONCENTRATION OF SOLUTION	DURATION OF DIPPING	KILL
	hours	*per cent*
0.5 per cent	1–3	0
1.0 per cent	1–3	0
1.5 per cent	1–3	0
0.125 per cent + 0.16 per cent acid*	1–3	0
0.25 per cent + 0.16 per cent acid	1	0
0.25 per cent + 0.16 per cent acid	2–3	33
0.25 per cent + 0.33 per cent acid	1	0
0.25 per cent + 0.33 per cent acid	2	33
0.25 per cent + 0.66 per cent acid	1	0
0.25 per cent + 0.66 per cent acid	2	100
0.25 per cent + 1 per cent acid	1	0
0.25 per cent + 1 per cent acid	2	100
0.5 per cent + 0.125 per cent acid	1	0
0.5 per cent + 0.125 per cent acid	2–3	100
0.5 per cent + 0.16 per cent acid	1–2	33
0.5 per cent + 0.16 per cent acid	3	100
0.5 per cent + 0.33 per cent acid	1	33
0.5 per cent + 0.33 per cent acid	2	100
0.5 per cent + 0.50 per cent acid	1	33
0.5 per cent + 0.50 per cent acid	2	100
0.5 per cent + 0.66 per cent acid	1	0
0.5 per cent + 0.66 per cent acid	2	100
0.5 per cent + 1 per cent acid	1	66
0.5 per cent + 1 per cent acid	2	100
1.0 per cent + 0.125 per cent acid	1	33
1.0 per cent + 0.125 per cent acid	2–3	100
1.0 per cent + 0.33 per cent acid	1–2	100
1.0 per cent + 0.66 per cent acid	1–2	100
1.0 per cent + 1 per cent acid	1–2	100
Water only (checks)	1–3	0

* 36 per cent acetic acid.

alcohol and sodium hydroxide begins at 77°F. and is completed on boiling. The reaction is as follows:

$$C_2H_5OCSSNa + H_2O = CS_2 + C_2H_5OH + NaOH.$$

The aqueous solution is decomposed without heating by acetic acid as follows:

$$C_2H_5OCSSNa + H_2O + HC_2H_3O_2 = CS_2 + C_2H_5OH + NaC_2H_3O_2 + H_2O.$$

The pure xanthate contains about 47 per cent carbon disulfide.

Table 2 gives the results of dipping *Popillia* grubs in various solutions of sodium ethyl xanthate.

It will be noted from table 2 that a 1.5 per cent solution of sodium ethyl xanthate (with no acid added) failed to kill *Popillia* grubs in 3 hours whereas a solution of 0.25 per cent xanthate plus $0.66\frac{2}{3}$ per cent acetic acid killed the grubs in 2 hours. This difference in killing properties between the xanthate alone and the xanthate plus acid is due to the fact that the xanthate without acid does not give off carbon disulfide except at higher temperatures, whereas with the xanthate plus acid combination the compound breaks up into carbon disulfide and alcohol and the resulting carbon disulfide kills the grubs.

Emulsions

Several mixtures of turpentine, castor oil, carbon disulfide and soap were compounded in various proportions for the purpose of obtaining an emulsion which would hold the carbon disulfide in suspension indefinitely. A good emulsion of this sort can be made according to the following formula: dissolve 4 cc. of carbon disulfide in 4 cc. of castor oil; add 4 cc. of turpentine; add 16 cc. of soap solution ($\frac{1}{2}$ pound of hard soap dissolved in 1 gallon of water). This emulsion did not separate into its component parts to the slightest degree at the end of 2 months. When the emulsion was added to water it remained in suspension for hours before the carbon disulfide began to settle to the bottom of the container.

Table 3 gives the results of dipping tests with this emulsion used at various strengths. It required a 2-hour exposure to a 1 per cent emulsion to give a 100 per cent kill, while the same results were obtained by a $\frac{1}{2}$-hour exposure to a 2 per cent emulsion. The killing properties of this emulsion are no doubt due to the carbon disulfide contained therein.

Miscellaneous dipping tests

Table 3 gives the results of dipping *Popillia* grubs in aqueous solutions of various compounds.

It will be observed that such compounds as copper sulfate, potassium fluoride and nicotine, all practically non-volatile compounds at ordinary temperatures, had a negligible effect on *Popillia* grubs. On the other hand, volatile compounds, the vapors of which are but slightly soluble in water,

such as carbon disulfide, thymol[2] and mustard oil, were decidedly toxic to the grubs.

The four sets of dipping tests detailed above indicate that *the only class of compounds in solution toxic to Popillia grubs are those capable of producing a*

TABLE 3

Results of dipping Popillia grubs in solutions of various compounds; grubs in the third (last) instar, and not embedded in soil

CONCENTRATION OF SOLUTION	DURATION OF DIPPING	KILL
		per cent
3.5 gm. sodium cyanide per gallon	10–110 min.	0
5.0 per cent sodium chloride	1–15 hrs.	0
5.0 per cent copper sulfate	15–30 min.	0
5.0 per cent copper sulfate	1 hr.	33
5.0 per cent copper sulfate	2 hrs.	66
5.0 per cent copper sulfate	3 hrs.	33
5.0 per cent copper sulfate	18 hrs.	0
5.0 per cent copper sulfate	24 hrs.	0
0.16 per cent nicotine	1–3 hrs.	0
1.0 per cent potassium fluoride	1–2 hrs.	0
5.0 per cent potassium fluoride	1–2 hrs.	0
10.0 per cent potassium fluoride	1–2 hrs.	0
Camphor (saturated solution)	1–2 hrs.	0
Carbon disulfide (saturated solution)*	15 min.	33
Carbon disulfide (saturated solution)*	30–120 min.	100
Thymol (saturated solution)†	1–2 hrs.	100
Mustard oil (saturated solution)‡	15 min.	66
Mustard oil (saturated solution)‡	30–60 min.	100
Chloroform (saturated solution)	15–45 min.	100
5 per cent of carbon disulfide emulsion§	1–2 hrs.	0
1 per cent of carbon disulfide emulsion§	2 hrs.	100
2 per cent of carbon disulfide emulsion§	½–1 hr.	100
3 per cent of carbon disulfide emulsion§	½–1 hr.	100
Water only (checks)	1–24 hrs.	0

* Approximately one part carbon disulfide in 1,000 parts water.

† See footnote below

‡ Allyl isothiocyanate (artificial), page 48.

§ See page 50.

gas insoluble or only slightly soluble in water. Substances such as copper sulfate, which in a 5 per cent solution reacts quickly on iron wire, has no appreciable effect on the grub.

[2] Thymol—a phenol found in volatile oil of thymus sp.; colorless, translucent, crystalline, 1 part soluble in 1,100 parts of water.

Mustard oil (allyl isothiocyanate, artificial). For a report of the above compounds when used as anthelmintics see Sollmann (3).

Dipping tests with grubs of Popillia japonica embedded in soil-balls

Plate 2 illustrates the apparatus employed in testing the various materials dissolved in water against the larvae in soil. The small wire cages shown in figure 4 were made of wire screen, (16 meshes to the inch), and were 1¾ inches long and ½ inch in diameter. Small cork stoppers, readily removable, were used to plug the ends. In using these cages a bit of soil was placed in the cage (holding it upright), then a healthy grub placed within, the cage filled with soil and stoppered. By maintaining the soil in a moist condition grubs could be kept in a normal condition in these small cages for many days or until they became restive and gnawed their way out through one of the cork stoppers.

Figure 1, plate 2, illustrates a wire cylinder 8 inches in diameter and 10 inches in height. Figure 2, plate 2 illustrates the same cylinder covered with a double thickness of muslin.

The object in using these two types of containers was to simulate as closely as possible the natural soil-ball. This subterfuge is necessary because it is impossible to obtain naturally the exact experimental condition required in this sort of work. The balled earth about the roots of a coniferous plant might or might not contain grubs and it is obvious that any examination made to determine the presence of grubs in such a soil-ball and their exact location therein would so disarrange the ball as to render it unfit for experimental purposes.

Under these circumstances it was necessary to resort to the use of artificial soil-balls. Such a ball was manufactured as follows. Soil was placed in the bottom of the cloth-covered cylinder and tamped down firmly to a depth of ½ inch. Three of the small wire cages described above, each containing a healthy grub, were then placed on top of the half-inch of soil in the large cylinder, one in the center and two within ½ inch of the sides. More soil was placed in the cloth-covered cylinder and tamped down to a total depth of 3¾ inches and another three grubs in small cages introduced as before. Again more soil to a total depth of 7½ inches was placed in the large cylinder and a third and final set of three grubs placed thereon and covered with ½ inch of soil and tamped down.

This arrangement gave a soil "ball" 8 inches in diameter and 8 inches in height containing 9 grubs, one in the center of the ball and eight distributed around the top, sides and bottom of the ball within ½ inch of the surface. By confining the grubs in these small wire cages they were held in the exact locations desired within the soil-ball while the muslin cover of the cylinder was comparable to the burlap bagging used in wrapping the soil-ball of a coniferous plant. In running a series of tests with a material it was a simple matter to make the required number of these soil-balls and to dip them, entirely submerged, in solutions contained in galvanized iron buckets or tubs.

The soil used throughout these tests consisted of a light sandy or garden loam. In these soil-ball tests the only soil condition which it seemed advis-

able to avoid—as indicated by the results—was excessively wet soil. Throughout this set of experiments the temperature of the soil-ball and of the solutions was recorded. These temperature records indicate the inadvisability of

TABLE 4

Results of dipping Popillia grubs, embedded in soil-balls, in aqueous solutions of sodium sulfocarbonate; grubs in third (last) instar

AMOUNT OF MATERIAL PER GALLON	DURATION OF DIPPING	KILL	SOIL TEMPERATURE	SOLUTION TEMPERATURE
		per cent	°F.	°F.
15 cc. sodium sulfocarbonate	30 min.	66.6	65–75†	85–79‡
15 cc sodium sulfocarbonate	60 min.	88.9	64–79	74–71
15 cc. sodium sulfocarbonate	120 min.	66.6	63–70	75–71
30 cc. sodium sulfocarbonate	15 min.	66.6	57–63	85–77
30 cc. sodium sulfocarbonate	30 min.	100.0	67–74	83–79
30 cc. sodium sulfocarbonate	60 min.	100.0	61–67	75–68
30 cc. sodium sulfocarbonate	120 min.	100.0	61–67	75–68
5 cc. sodium sulfocarbonate plus 20 cc. sodium carbonate	60 min.	22.2	79§	75¶
10 cc. sodium sulfocarbonate plus 20 cc. sodium carbonate	60 min.	55.5	80	75
15 cc. sodium sulfocarbonate plus 20 cc. sodium carbonate	60 min.	55.5	80	75
30 cc. sodium sulfocarbonate plus 20 cc. sodium carbonate	60 min.	88.9	80	75
5 cc. sodium sulfocarbonate in H_2O charged with CO_2.	60 min.	0	68–69	76–75
10 cc. sodium sulfocarbonate in H_2O charged with CO_2.	60 min.	66.0	68–69	76–75
15 cc. sodium sulfocarbonate in H_2O charged with CO_2.	60 min.	88.9	68–69	76–75
5 cc. sodium sulfocarbonate plus 8 cc. acetic acid*	60 min.	11.1	63–73	76–73
10 cc. sodium sulfocarbonate plus 8 cc. acetic acid*	60 min.	33.3	63–73	76–73
15 cc. sodium sulfocarbonate plus 8 cc. acetic acid*	60 min.	33.3	63–73	76–73
10 cc. sodium sulfocarbonate plus 10 cc. acetic acid	24 hrs.	0	81	65
10 cc. sodium sulfocarbonate plus 15 cc. acetic acid	1 hr.	55.5	71	75
20 cc. sodium sulfocarbonate plus 20 cc. acetic acid	1 hr.	66.0	71	75
25 cc. sodium sulfocarbonate plus 25 cc. acetic acid	1 hr.	88.9	71	75
Water only (check)	1–24 hrs.	0	As above	As above

* 36 per cent acetic acid.

† Initial temperature of soil ball 65° F., raised to 75° as a result of dipping in solution with initial temperature of 85°.

‡ Initial temperature of solution 85° F., reduced to 79° by cooling effect of soil-ball with initial temperature of 65°.

§ Initial temperature of soil-ball.

¶ Initial temperature of solution.

allowing the soil or dip to run below 60° while 70° to 75° is a good working temperature from the standpoint of both results and ease in handling materials.

The results of these dipping tests with soil-balls containing *Popillia* grubs are given in tables 4 and 5. The dipping of soil-balls in a solution of 30 cc. of sodium sulfocarbonate to the gallon of water for a period of 30 minutes

gave 100 per cent control; the grubs were dead at the end of 5 days, during which time the soil-ball had remained undisturbed at a room temperature of 70°F. During these 5 days the sodium sulfocarbonate taken up by the soil-ball while in the solution reacted with the carbon dioxide in the soil, giving off carbon disulfide in sufficient quantity to produce 100 per cent mortality.

The dipping of soil-balls in a solution containing 30 cc. of sodium sulfocarbonate and 20 gm. of sodium carbonate per gallon did not give 100 per

TABLE 5

Results of dipping Popillia grubs, embedded in soil-balls, in aqueous solutions of various compounds; grubs in third (last) instar

SOLUTION	DURATION OF DIPPING	KILL	SOIL TEMPERATURE	SOLUTION TEMPERATURE
		per cent	°F.	°F.
0.25 per cent sodium ethyl xanthate	24 hrs.	11.1	80†	78‡
1.5 per cent sodium ethyl xanthate	1 hr.	77.8	80	78
0.5 per cent sodium ethyl xanthate plus 0.5 per cent acetic acid*	1–2 hrs.	66.0	73	69
1 per cent sodium ethyl xanthate plus 1 per cent acetic acid*	1 hr.	100.0	73	69
1.5 pe cent sodium ethyl xanthate plus 1.5 per cent acetic acid*	1 hr	100.0	73	69
Saturated solution of hydrogen sulfide	1 hr.	33.3	68	75
Saturated solution of carbon disulfide	15 min.	11.1	80	75
Saturated solution of carbon disulfide	60 min.	0	79	75
Saturated solut'on of carbon disulfide	120 min.	0	79	75
Saturated solution of carbon disulfide	24 hrs.	0	81	75
Saturated solution of CS_2 and H_2S	1 hr.	0	60	75
2 per cent "solution" of carbon disulfide emulsion	1 hr.	44.4	79	75
3 per cent "solution" of carbon disulfide emulsion	1 hr.	33.3	79	75
Thymol (saturated solution)	1 hr.	22.3	79	78
Thymol (saturated solution)	6 hrs.	33.3	79	78
Thymol (saturated solution)	12 hrs.	22.3	79	78
Thymol (saturated solution)	24 hrs.	11.1	79	78
Mustard oil (saturated solution)	24 hrs.	66.6	79	78
Saturated solution of mustard oil and CS_2	24 hrs.	66.6	79	78
Water only (checks)	1–24 hrs.	0	As above	As above

* 36 per cent acetic acid.

† Temperature of soil-ball before dipping.

‡ Temperature of solution at beginning of dipping period.

cent mortality when examined 5 days later. This is explained by the fact that the sodium carbonate reacted with any carbon dioxide present in the soil (forming sodium bicarbonate, $NaHCO_3$, viz.: $Na_2CO_3 + H_2O + CO_2 = 2NaHCO_3$) thereby preventing the decomposition of the sodium sulfocarbonate into carbon disulfide.

Nothing apparently was gained by the addition of acetic acid to solutions of sodium sulfocarbonate employed in dipping soil-balls containing *Popillia*

grubs; acetic acid caused a much more rapid decomposition of the sulfocarbonate with consequent evolution of carbon disulfide, but the same mortality was secured by using an equal concentration of sulfocarbonate without acid, indicating that the carbon dioxide occurring naturally in the soil is sufficient in quantity to decompose the sulfocarbonate.

Table 5 gives the results of dipping *Popillia* grubs, embedded in soil-balls in aqueous solutions of various compounds. The dipping of soil-balls in solutions of sodium ethyl xanthate gave an indifferent grub mortality due to the fact that the material was not apparently acted upon by the weak concentration of carbonic acid in the soil. A combined solution of 1 per cent xanthate and 1 per cent acetic acid gave 100 per cent mortality. In this case the carbon disulfide formed by the reaction of the acid and the xanthate was the active killing agent.

TABLE 6

Comparison of results obtained in dipping Popillia grubs embedded and not embedded in soil, in solutions of various compounds

SOLUTION	POPILLIA GRUBS NOT EMBEDDED IN SOIL		POPILLIA GRUBS EMBEDDED IN SOIL-BALLS	
	Duration of dipping	Kill	Duration of dipping	Kill
		per cent		*per cent*
0.5 per cent sodium ethyl xanthate plus 0.5 per cent of 36 per cent acetic acid	2 hrs	100	2 hrs.	66.0
15 cc. sodium sulfocarbonate plus 15 cc. of 36 per cent acetic acid per gallon	30 min.	100	1 hr.	55.5
Carbon disulfide (saturated solution)	30 min.	100	24 hrs.	0
2 per cent of carbon disulfide emulsion	30 min.	100	1 hr.	33.0
Saturated solution of thymol	60 min.	100	24 hrs.	11.1
Mustard oil (saturated solution)	30 min.	100	24 hrs.	66.6

The dipping of soil-balls containing *Popillia* grubs in saturated aqueous solutions of carbon disulfide, thymol and mustard oil and a 3 per cent solution of carbon disulfide emulsion all resulted in an inferior kill.

A comparison is given in table 6 of the results obtained in dipping *Popillia* grubs, embedded in soil-balls and *not* embedded in soil, in solutions of various compounds. When, for instance, *Popillia* grubs *not* embedded in soil-balls were dipped in saturated solutions of carbon disulfide, thymol, mustard oil, etc., the grubs were dead in 30 minutes. When however the grubs were embedded in soil-balls and the soil-balls dipped in the same strength of the above solutions for 24 hours the grubs were unharmed.

The question naturally arises as to why this class of slightly soluble compounds, comprising carbon disulfide, thymol, mustard oil, etc., so effective in killing *Popillia* grubs when the latter are *not* embedded in soil, should so signally fail to kill the grubs when they *are* embedded in soil. The following

tests would seem to indicate the reason for this dissimilarity in results. In making these tests a solution was allowed to percolate through the soil-column of sandy loam contained in the drain pipe as shown in plate 2, figure 3. This soil-column was 10 cm. in diameter and 20 cm. in height, giving a total soil-volume of approximately 1,570 cc. The percolate was collected at the bottom of the soil-column by means of the funnel and beaker.

Using the above apparatus, a saturated solution of thymol was gradually poured onto the top of the soil-column and allowed to percolate through the soil, the liquid being collected as it drained away from the column into the beaker. The first 600 cc. of the percolate gave no taste or odor of thymol, the next 100 cc. contained a trace of thymol and the percolate gradually increased in concentration of thymol until 3800 cc. had been collected. At this point a sample of the percolate contained virtually the same concentration of thymol as the solution introduced into the top of the soil-column. Substantially the same results were obtained with individual saturated solutions of carbon disulfide, mustard oil and chloroform; in each case no trace of the solute could be detected in the percolate until several hundred cubic centimeters had drained through the column and the concentration of the substance in the percolate was not equal to the concentration of the saturated solution until several thousand cubic centimeters had drained through the column. *In other words the material in solution is largely "filtered out" by the soil until the latter becomes saturated, whereupon the solution percolates through unchanged.* The exact cause of this removal of solute by soils cannot be specifically stated but is due in all probability to one or all of three causes, namely, soil absorption, soil adsorption, and chemical action between certain of the soil constituents. The last named phenomenon may be responsible for the removal of *some* materials when their solutions are allowed to percolate through soils, but the scope of chemical action in the soil is not broad enough to account for the almost automatic removal of practically everything in solution which is allowed to percolate through soil. The phenomenon would seem to be physical rather than chemical and in all probability the removal of the solute may be attributed in large part to the adsorption of the material in solution by the moisture film enveloping the minute soil particles.[3]

[3] Detailed investigations by various soil physicists have shown that the individual moisture-films surrounding these minute soil particles exist or are held under comparatively tremendous force or pressure. Instances have been recorded of a pressure of 6,000 to 25,000 atmospheres. This pressure or compression results in a pronounced liquid density of the moisture-film, consequently there is a decidedly greater *quantity* of liquid present in the films than would be the case if it were under a pressure of only one atmosphere. Since there is more liquid present in the film it follows that this compressed liquid can dissolve or (absorb) more of a given solute than could the same *volume* of liquid under normal conditions of pressure, etc. Under these circumstances when a solution is introduced into the soil it percolates through the pore space where it comes in contact with the films surrounding the soil particles and these moisture films absorb the solute to the limit of their capacity.

This would seem to indicate that when solutions of soil insecticides are added to soil the material in solution is adsorbed up to a point governed by the capacity of the moisture films surrounding the soil particle. The experimental data also indicate that that portion of the toxic solute adsorbed by the soil particles is largely rendered *impotent* as far as its ability to act as a killing agent against insects in the soil is concerned. *Apparently the unadsorbed portion of the toxic solute is responsible for any mortality of soil insects resulting from the use of the solutions.* The quantity of solute remaining unabsorbed in the soil, when a given quantity of solution is added to a given quantity of soil, will depend upon the *initial* strength of the solution.

The limitations which this phenomenon of soil adsorption imposes upon the process of dipping soil-balls in solutions toxic to insects are evident when one considers the inter-action of the soil-ball and the dip. When a ball of earth wrapped in burlap is submerged in a liquid, the liquid penetrates into the soil-ball, largely displacing the air from the pore-space of the soil. *When the liquid has filled the soil pore-space, the movement of liquid into the soil-ball practically ceases.*

If the above liquid is a solution the adsorption of the solute by the soil particles goes on while the liquid is penetrating into the soil-ball and continues for some time after this penetration is complete and the liquid within and without the soil-ball is in a state of relative rest. It is obvious that the amount of the solute finally remaining unadsorbed in the liquid at rest within the soil-ball will depend absolutely on the initial concentration of the solution. Relatively dilute solutions of toxic compounds are largely adsorbed, leaving little unadsorbed material physically free to produce insect mortality. This explains the failure of saturated solutions of carbon disulfide, thymol, etc., to produce mortality of *Popillia* grubs embedded in soil-balls, although such solutions kill the grubs readily enough when the latter are not embedded in soil. *The saturated solutions of these materials are relatively dilute and the solute is largely adsorbed.*

Of the various compounds tested only two produced 100 per cent mortality of *Popillia* grubs when the latter were embedded in soil.[4] These two compounds were sodium sulfocarbonate and sodium ethyl xanthate. The positive results obtained with these two compounds are due to the fact that, unlike carbon disulfide, they are soluble in water in all proportions. By dipping *Popillia* grubs embedded in soil-balls in sufficiently concentrated

[4] The soil-balls used for these dipping tests contained 9 grubs (see page 50 for description of these balls). One grub was placed in the center of the ball and the remaining 8 distributed around the top, bottom and sides of the ball, within ½ inch of the surface. The grub in the center of the ball invariably escaped the action of the toxic gas when comparatively dilute dipping solutions were used, although one or more of the grubs located near the surface of the ball might succumb. It was only by using comparatively concentrated solutions that the grub in the center of the ball could be killed, in which case, as a matter of course, the grubs near the surface of the ball succumbed also.

solutions of these materials—for instance, 30 cc. of sodium sulfocarbonate to the gallon—an amount of the material sufficient to produce 100 per cent mortality of the grubs remains unadsorbed in the soil.[5]

The grubs in the soil are killed to some extent by contact with the sodium sulfocarbonate itself, but the efficiency of the material is due largely to the formation of carbon disulfide by the action of organic acids. The toxicity of sodium ethyl xanthate is entirely due to its decomposition into carbon disulfide by organic acids.

DOSAGE TESTS WITH BALLED CONIFEROUS PLANTS

Plate 1 illustrates the type and size of coniferous plants employed in the dosage tests outlined in table 7. The plants varied from 8 to 12 inches in height and were balled and wrapped as for shipment before they were dipped in the various solutions. In dipping, the ball of earth was completely submerged in the solution and allowed to remain undisturbed throughout the duration of the dipping period. Galvanized-iron tubs or buckets were used as containers for the solutions. On removal from the solution the plants were allowed to drain for 3 days in a room maintained at a temperature of 70°F. and were then planted in a small experimental nursery and kept under observation during the remainder of the season.

Table 7 gives the results of dipping coniferous plants in various solutions of three compounds, namely, sodium cyanide, sodium sulfocarbonate and sodium ethyl xanthate. Sodium cyanide in dilute solutions was extremely toxic to the plant, while the compound gave poor results against *Popillia* grubs embedded in soil. Sodium sulfocarbonate when used at the rate of 30 cc. per gallon of water, a dose toxic to *Popillia* grubs embedded in soil, shattered the roots of the plants and the tops were completely browned in from 10 to 15 days. The table indicates the results with smaller doses of this compound. In the case of trees treated with 10 cc. of sodium sulfocarbonate per gallon on May 20, the foliage was unimpaired but an examination of the root system 10 days after the treatment disclosed the fact that the root tips had been killed. Twenty days after treatment new root tips had formed and the tree resumed growth. However, during the season the tree made only one-half the growth of the check trees, indicating a slowing down as a result of the treatment with sodium sulfocarbonate.

Sodium ethyl xanthate, when used at a strength capable of killing *Popillia* grubs embedded in soil, damaged the tree in virtually the same manner as did the sodium sulfocarbonate.

[5] Various tests conducted during the course of the above experiments indicate that the larvae of *Popillia japonica* are more resistant to toxic compounds than are other similar soil-inhabiting larvae such as *Lachnosterna*. Sasscer and Sanford (2) found that the larvae of *Popillia japonica* were the most difficult to kill of the various larvae subjected to the gas.

DATE	MATERIAL EMPLOYED	CONCENTRATION OF SOLUTION	DURATION OF DIPPING	SOIL TEMPERATURE	SOLUTION TEMPERATURE	EFFECT OF SOLUTION ON PLANTS
				°F.	°F.	
May 5	Sodium cyanide	3.5 gm. per gal.	45 min.	44–70	90–78	Dead in 12 days*
May 2	Sodium cyanide	7.0 gm. per gal.	45 min.	44–70	90–78	Dead in 6 days
May 2	Water only (checks)	Check	45 min.	44–70	90–78	Made vigorous growth during season
May 20	Sodium sulfocarbonate	10.0 cc. per gal.	15 min.	64–70	74–72	Made one-half normal growth during season
May 20	Sodium sulfocarbonate	15.0 cc. per gal.	15 min.	64–71	75–73	No growth during season
May 20	Sodium sulfocarbonate	20.0 cc. per gal.	15 min.	64–71	75–73	1 dead in 20 days, 2 made no growth during season
May 20	Sodium sulfocarbonate	30.0 cc. per gal.	15 min.	64–73	76–75	Dead in 10 days
May 20	Water only (checks)	Check	15 min.	64–71	75–73	Made vigorous growth during season
July 6	Sodium sulfocarbonate	15.0 cc. per gal.	1 hr.	77	76	Dead in 20 days
July 6	Sodium sulfocarbonate	30.0 cc. per gal.	1 hr.	77	76	Dead in 15 days
July 6	Sodium sulfocarbonate 36 per cent acetic acid	15.0 cc. per gal. 15.0 cc. per gal.	1 hr.	76	75	Trees badly shattered; will die
July 6	Sodium sulfocarbonate 36 per cent acetic acid	20.0 cc. per gal. 20.0 cc. per gal.	1 hr.	71	75	Dead in 15 days
July 6	Sodium sulfocarbonate 36 per cent acetic acid	25.0 cc. per gal. 25.0 cc. per gal	1 hr.	77	76	Dead in 15 days
July 6	Sodium sulfocarbonate 36 per cent acetic acid	30.0 cc. per gal. 30.0 cc. per gal.	1 hr.	71	75	Dead in 15 days
July 6	Sodium ethyl xanthate 36 per cent acetic acid	20.0 cc. per gal. 20.0 cc. per gal.	1 hr.	77	76	Trees badly shattered; will die
July 6	Sodium ethyl xanthate 36 per cent acetic acid	40.0 cc. per gal. 40.0 cc. per gal.	1 hr.	77	76	Dead in 20 days
July 6	36 per cent acetic acid	20 cc. per gal.	1 hr.	77	76	Trees injured slightly
July 6	Water only (checks)	Check	1 hr.	77	76	Made vigorous growth during season

* The conifers used for tests made May 20 were *Thuya vervaenaena*, all remaining tests were made with *Retinospora Pisifera aurea.*

SUMMARY

Dipping tests indicate that certain compounds in solution, capable of producing a gas insoluble or only slightly soluble in water, are toxic to *Popillia* grubs. These compounds may be divided into two classes:

I. Compounds slightly soluble in water, e.g., carbon disulfide, thymol, mustard oil, etc.

II. Compounds readily soluble in water such as sodium sulfocarbonate and sodium ethyl xanthate. These compounds in solution, on being decomposed by organic acids yield cardon disulfide, the active killing agent.

Saturated solutions of compounds in class I (about 1 to 1,000) readily kill *Popillia* grubs when the latter are removed from the soil and dipped in the solution for a definite period of time. However, when *Popillia* grubs are embedded in a soil-ball and the latter dipped in these solutions the grubs contained within the soil-ball remain unharmed. Soil adsorption, or in other words physical "locking up" of the compound in solution by the moisture film surrounding the minute soil particles, is the apparent reason for the failure of these relatively dilute solutions to function in soil. That portion of the compound adsorbed by the soil is apparently rendered impotent as far as its ability to produce grub mortality in the soil is concerned.

Compounds of class II when used in dilute solutions give results comparable to those obtained in the employment of compounds in class I. However, when compounds of class II are employed in relatively concentrated solutions, a quantity of the compound sufficient to produce 100 per cent mortality of *Popillia* grubs remains free in the soil after the soil particles have adsorbed the compound to the limit of their capacity.

The comparatively concentrated solutions of sodium sulfocarbonate and sodium ethyl xanthate, when used for the treatment of balled earth about the roots of coniferous plants for the control of Japanese beetle larvae, injure the plants to an extent which prohibits the use of these compounds in practice.

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(2) Sasscer, E. R., and Sanford, H. L. 1918 Effect of hydrocyanic-acid gas under vacuum conditions on subterranean larvae. *In* Jour. Agr. Res., v. 15, no. 3, p. 133–136.

(3) Sollmann, Torald 1918 Anthelmintics: Their efficiency tested on earthworms. *In* Jour. Pharm. Exp. Ther., v. 12, no. 3, p. 129.

PLATE 1

Fig. 1. Small coniferous plant with ball of earth about the roots; the plant has just been taken from the nursery row.

Fig. 2. Same plant as in figure 1, but wrapped in burlap for shipment.

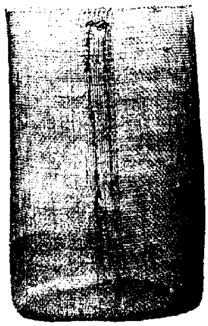
PLATE 2

FIG. 1. Wire cylinder 8 inches in diameter and 10 inches in height with wire bottom.

FIG. 2. Same cylinder as in figure 1, covered with double thickness of muslin; employed as container for artificial soil-ball.

FIG. 3. Apparatus employed in studying the percolation of toxic solutions through soil.

FIG. 4. Small wire cage, with removable cork stoppers, used as containers for *Popillia* grubs when embedded in soil-balls.



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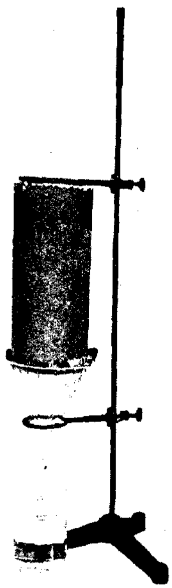

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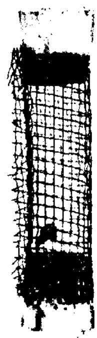

4

EXPERIMENTS WITH HOT WATER IN THE TREATMENT OF BALLED EARTH ABOUT THE ROOTS OF PLANTS FOR THE CONTROL OF JAPANESE BEETLE LARVAE

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One of the experimental problems in connection with the control of the green Japanese beetle (*Popillia japonica* Newm.) consists in finding a method of killing *Popillia* grubs which may possibly occur in balled earth about the roots of coniferous and certain other ornamental plants or in the soil in which potted plants are grown. Such a method is needed because there are several nurseries growing these classes of plants within the area in New Jersey and Pennsylvania infested by the beetle and the marketing of their product is regulated by state and federal quarantines. Among other experiments (1) carried out during 1920 on this phase of the Japanesse beetle problem the writer tested hot water (100° to 130°F.) in order to determine its value as a practical control measure for the treatment of balled earth.

NURSERY PRACTICE IN SHIPPING CONIFEROUS PLANTS

In order that coniferous plants, such as *Arbor vitae* spruces, pines, etc., may be successfully transplanted, it is essential that the tree be dug in such a way that a sufficient proportion of the roots and rootlets are not disarranged in the soil or exposed to the drying action of the atmosphere. In order to secure a good soil-ball about the roots, nurserymen transplant conifers at frequent intervals in the nursery row. This frequent transplanting, with the accompanying root-pruning, results in a compact mass of fibrous roots in the immediate vicinity of the base of the tree. This compact mass of roots and soil, or the *soil-ball* as it is called, facilitates handling and insures successful transplanting. As an added precaution in shipping this class of stock the soil-ball is wrapped in burlap.

BASIS OF THE EXPERIMENTAL WORK

As stated above, this class of nursery stock when ready for shipmeht is tightly wrapped in burlap bagging. Under these circumstances the plants can be dipped or soaked in water with impunity as far as mechanical injury is concerned, since only a very small proportion of the soil escapes through the bagging and the relative position of the roots within the soil-ball is undisturbed. On removal from the liquid the plants would be ready for shipment after being allowed to drain for a few hours.

EXPERIMENTAL PROCEDURE

Aside from the few initial experiments the following steps were taken in testing hot water:

1. *Popillia* grubs were dipped in water at varying temperatures for varying periods of time in order to determine their resistance.

2. *Popillia* grubs, *embedded in soil-balls,* were subjected to the action of heated water.

3. Heated water was tested as to its action on the plant; small coniferous plants and azaleas, both with balled earth about their roots, were employed.

DIPPING TESTS WITH GRUBS OF POPILLIA JAPONICA

Tests were made in order to determine the degree of resistance of the grub when dipped in water at varying temperatures. The apparatus employed consisted of a small gas-burner, two galvanized-iron tubs, the smaller having about one-half the capacity of the larger, and a pair of long chemical Fahrenheit thermometers. The large tub was placed on the gas range and partially filled with water so that the small tub half-filled with water floated in the large tub. This arrangment is comparable to a water bath or double-boiler on a large scale.

On applying heat the water in the large tub slowly increased in temperature and also warmed up the water in the small tub. By adjusting the flow of gas it was possible to keep the water in the large tub at a fairly even temperature while the water in the small tub could be kept at a slightly lower and fairly constant temperature.

When the water in the small tub had remained at the desired temperature for half an hour, thereby indicating that the fluctuation in temperature was negligible from an experimental standpoint, the grubs, confined in small wire cages, were introduced into the water. After being subjected to the water for a definite period of time they were removed and placed on moist soil in tin salve-boxes for subsequent observation.

These tests indicate that, under the above experimental conditions, third-instar *Popillia* grubs have a certain resistance to water at various temperatures as follows.

Popillia grubs are killed by an exposure of 1 to 2 minutes in water at 130°F., 3 to 4 minutes in water at 125°F., and 6 to 8 minutes at 120°. One cannot bear the hand in water at these temperatures for any length of time.

The grubs are killed by an exposure of 12 minutes in water at 115°F. One can hardly bear the hand in water at this temperature.

The grubs are killed by an exposure of 45 minutes in water at 110°F. Water at this temperature is barely comfortable to the hand.

The grubs withstand an exposure of 2 hours in water at 105°. Water at this temperature is just comfortable to the hand.

Popillia grubs withstand an exposure of 24 hours in cold water (50°F.).

EXPERIMENTS WITH POPILLIA GRUBS EMBEDDED IN SOIL

A *soil-ball* 8 inches in diameter was prepared from garden loam. During the process of manufacture 6 grubs, each in a small individual wire cage (1¾ inches long and ½ inch in diameter), were placed in the ball, two in the center and the remaining four within an inch of the surface. The ball was then wrapped in burlap and placed in water at the desired experimental temperature, the apparatus described above being employed.

Several tests at varying water temperatures were made of which the test represented graphically in figure 1 is representative. The curve expressed by the upper line represents the gradation in the temperature of the water from an initial temperature of 110°F. (the temperature when the soil-ball was introduced). The lower curve represents the gradation in temperature of the center of the soil-ball during its exposure to the heated water.

The soil-ball, at the moment of introduction into the water heated to 110°, had a temperature of 51°F. Five minutes afterward the temperature of the center of the ball had jumped to 81°F., while the temperature of the water surrounding the ball had dropped to 105°F. The increase of 30° in the temperature of the soil-ball (measured at the center) is due to the inrush of the warm water into the air-spaces of the soil. The water surrounding the ball is cooled from 110° to 105° by imparting heat to the ball.

Under these circumstances, 5 minutes after the soil-ball had been placed in the water the temperature of the water had fallen to 105° and the temperature of the ball (*this included the water taken up by the ball*) had risen to 81°. An examination of the graph shows that during the next 65 minutes (the heat having remained constant) the temperature of the soil-ball gradually creeped up to 101°F. while the water surrounding the ball remained constant at 105°.

During the next 75 minutes the soil-ball and surrounding water both increased in temperature and at the end of this period the surrounding water had attained its initial temperature of 110°. However, at this time the soil-ball still registered only 106° and 20 minutes elapsed before the center of the soil-ball registered 110°. During these 20 minutes it was necessary to decrease slightly the amount of heat in order to prevent the temperature of the water surrounding the ball from going above 110°.

It therefore required 160 minutes to bring the soil-ball up to the desired temperature of 110° and the ball was continued in the water at this temperature for an additional 45 minutes, at the end of which period it was removed and allowed to drain.

While draining, the surface of the ball cooled rapidly but the center persisted at a temperature of 110° for 15 minutes and then slowly cooled to room temperature as indicated in figure 1.

On the cooling of the soil-ball to room temperature the grubs were removed for examination. Tests conducted in the fashion described above gave the following results.

When a soil-ball containing *Popillia* grubs is placed in water at 110°, the ball brought up to a temperature of 110° and maintained at 110° for 45 minutes, the grubs all die. If the temperature is maintained in the same way at 105° only 66 per cent of the grubs succumb. At 100° only 33 per cent succumb and below this temperature they are not affected.

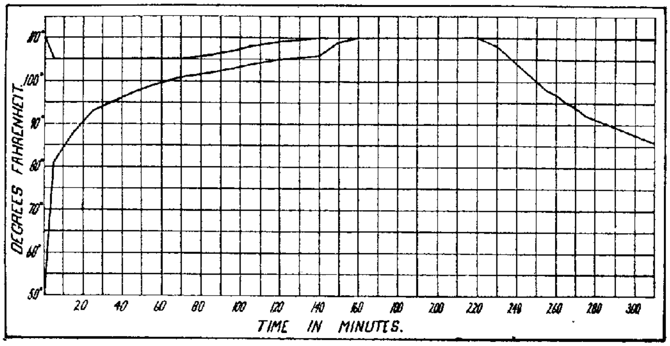

FIG. 1. RANGE AND VARIATION IN TEMPERATURE OF SOIL-BALL AND WATER BATH

TREATMENT OF BALLED-PLANTS WITH HEATED WATER

Two species of plants, which in practice are shipped with earth about their roots, were treated with heated water. In these tests the plants were placed in the water at the desired temperature, the ball of earth containing the roots allowed to warm up to that temperature and then maintained in the water at that temperature for 45 minutes. They were then removed from the water and allowed to drain for 2 days at room temperature before being planted in the nursery row. These treated plants were kept under observation during the remainder of the season.

Results with Azalea amoena

The balls of earth containing the roots of these plants were about 8 inches in diameter.

Plants treated as above at a temperature of 120°F. were checked decidedly in their growth during the season.

Plants treated at a temperature of 115°F. were appreciably checked in their growth during the season.

Plants (checks) treated at tap-water temperatures made normal growth during the season.

A few days after the treatment of these plants the roots were carefully examined for injury. No evidence of injury could be detected in roots large

enough to cross-section by hand but the subsequent growth of the plants during the season indicated that the small feeding rootlets were injured by the treatment.

Results with coniferous plants

The plants employed in these tests were *Retinospora Pisifera aurea*, a variety of *Arbor vitae.* The ball of earth containing the roots was about 8 inches in diameter.

Plants treated at a temperature of 110°F. were badly injured. Some died and in others half the foliage was dead at the end of the season. The main roots were injured severely. Cells throughout the entire cross-section of these roots were killed.

Plants treated at a temperature of 105°F. were decidedly affected. The roots were injured and the foliage made practically no growth during the season.

Plants treated at a temperature of 100°F. were affected to some extent by the treatment. The small rootlets were killed and the top did not begin to send out new foliage until late in the season.

ANALYSIS OF RESULTS

The experimental data outlined above clearly indicate that the roots of plants are less capable of withstanding exposure to heated water than are the larvae of *Popillia japonica.* When roots are exposed to water at the minimum temperature necessary to insure the mortality of the grubs the roots are literally "cooked" with the result that the plant succumbs or its subsequent growth is at least decidedly checked.

Aside from the question of injury to the plant the treatment would not seem to lend itself to actual practice due to the slowness of the method and difficulty of controlling the temperature of the water. This last fact is evident when one considers the problem of heat radiation from the water-container resulting in a variation of several degrees in temperature between the bottom, sides and center of the mass of water. The slowness of the method is evident when one considers that it requires more than 3 hours to treat a small ball of earth.

SUMMARY AND CONCLUSIONS

The third-instar grubs of *Popillia japonica* withstand an exposure of 2 hours in water heated to a temperature of 105°F.; they succumb to an exposure of 45 minutes in water at a temperature of 110°F., to an exposure of 12 minutes at 115°F., to an exposure of 6 to 8 minutes at 120°F., to an exposure of 3 to 4 minutes at 125° and to an exposure of 1 to 2 minutes at 130°F.

When a bath of heated water (minimum temperature 110°F.) is used as a method of killing *Popillia* grubs in the balled-earth about the roots of plants

the soil-ball must be immersed until brought up to a temperature of 110°F. and remain immersed at that temperature for a period of 45 minutes. The process of warming up the soil is necessarily slow because it is not safe to allow the temperature of the water-bath to exceed 110°F. at any time. It requires 3 hours and 25 minutes to treat a soil-ball 8 inches in diameter by this method. Under the circumstances, and aside from the consideration of injury to the plant, the method is too slow to be utilized to any extent in practice.

The results indicate that plants vary considerably in their resistance to immersion in heated water for various periods of time at various temperatures, but that even the more resistant plants are affected and checked in their subsequent growth.

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'THE RELATION OF THE HYDROGEN-ION CONCENTRATION OF NUTRIENT SOLUTIONS TO GROWTH AND CHLOROSIS OF WHEAT PLANTS

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INTRODUCTION

In a previous paper (4) the writers published the results of an investigation of the hydrogen-ion concentration of the three-salt nutrient solutions recommended (3) by a special committee of the Division of Biology and Agriculture of the National Research Council. These results indicated that none of the differences in the growth rate of wheat plants in sand cultures with any one type of solution could be attributed to variations in the hydrogen-ion concentration of the nutrient solutions. This, however, does not preclude the possibility that the relative merit of the six types of solutions is influenced to some extent by their respective ranges of H-ion concentrations. Table 1, summarized from the previous paper (4), shows the magnitude of the pH ranges.

TABLE 1

Composition and pH ranges of the three-salt solutions

TYPE	CALCIUM SALT	POTASSIUM SALT	MAGNESIUM SALT	pH RANGE
I	$Ca(NO_3)_2$	KH_2PO_4	$MgSO_4$	4.4–4.8
II	$Ca(NO_3)_2$	K_2SO_4	$Mg(H_2PO_4)_2$	3.5–3.8
III	$Ca(H_2PO_4)_2$	KNO_3	$MgSO_4$	3.5–4.1
IV	$Ca(H_2PO_4)_2$	K_2SO_4	$Mg(NO_3)_2$	3.5–4.1
V	$CaSO_4$	KNO_3	$Mg(H_2PO_4)_2$	3.5–4.3
VI	$CaSO_4$	KH_2PO_4	$Mg(NO_3)_2$	4.8–5.3

The types containing potassium phosphate (KH_2PO_4) have a lower H-ion concentration than those containing either magnesium phosphate, ($Mg(H_2PO_4)_2$) or calcium phosphate, ($Ca(H_2PO_4)_2$). It may be added that, in general, within any one type, the H-ion concentration of the solution is a function of the volume-molecular proportion of the di-hydrogenphosphate salt present. These considerations, together with the existence of only a very limited amount of work bearing on the relation of the H-ion concentration of nutrient solutions to the growth of the higher plants, suggested to the authors the desirability of undertaking the work which is described in this paper.

PLAN OF THE EXPERIMENT

Wheat was grown in sand (3) cultures for a period of 2 months. The nutrient solutions were changed twice a week. Four different solutions were used as the basis of this experiment. Each basic solution was modified by the additions of 0.025 M potassium sulfate, 0.025 M sulfuric acid or 0.05 M potassium hydroxide[1] at the rate of 20 cc. to each liter of nutrient solution. Potassium hydroxide and sulfuric acid were added to vary the H-ion concentration while potassium sulfate was added to check up the effect of the extra potassium or sulfur added in the form of potassium hydroxide or sulfuric acid. Each basic solution was modified in this manner so as to have three distinctly different pH values without materially altering the relative concentration of the six essential ions. The pH values of the solutions thus produced varied from 3.06 to 7.00.

The basic solutions used were I R_3S_3, III R_3S_3, as described by a special committee of the National Research Council (3), and two concentrations of a special solution so made up as to contain the essential ions in approximately the same proportion as they are found in a normal wheat plant. The stock solution from which this solution was made had the following composition:

	grams per liter
Calcium phosphate ($Ca(H_2PO_4)_2$)	4.10
Calcium nitrate ($Ca(NO_3)_2$)	18.00
Potassium nitrate (KNO_3)	7.80
Magnesium nitrate ($Mg(NO_3)_2$)	0.20
Magnesium sulfate ($MgSO_4$)	0.75
Sodium chloride (NaCl)	0.10

This stock solution was diluted 1 to 12 and 1 to 24. When diluted 1 to 12 a nutrient solution was produced having an osmotic pressure of 1.06 atmospheres. The above solutions, for convenience, will be referred to as I R_3S_3, III R_3S_3, Special 1–12, and Special 1–24.

DATA AND DISCUSSION

The data upon which the discussion is based are given in table 2. This table also furnishes the basis for the curves shown in figure 1. The outstanding feature of the results is the very marked influence of the H-ion concentration upon growth and chlorosis. The physico-chemical properties of the solutions themselves are very interesting and will be given consideration under a separate heading.

[1] In terms of normality 0.025 M sulfuric acid and 0.05 M potassium hydroxide are equivalent, each having a reacting power equivalent to 0.05 N solutions.

TABLE 2

Weight of tops and appearance of wheat plants grown in sand cultures with nutrient solutions having different hydrogen-ion concentrations

CULTURE NUMBER	BASIC SOLUTIONS	20 CC. ADDITIONS TO EACH LITER OF BASIC SOLUTION	HYDROGEN-ION CONCENTRATIONS OF NUTRIENT SOLUTION	GREEN WEIGHT OF TOPS (5 PLANTS)	AVERAGE GREEN WEIGHT	APPEARANCE OF PLANTS FROM 2 WEEKS AFTER PLANTING TO HARVEST
			pH	*gm.*	*gm.*	
1 2	I R_1S_3	None	4.60	14.0 13.8	13.9	Very chlorotic
3 4		0.025 *M* K_2SO_4	4.68	18.0 14.8	16.4	Very chlorotic
5 6		0.025 *M* H_2SO_4	3.26	36.0 31.2	33.6	Green
7 8		0.05 *M* KOH	5.83	7.5 9.1	8.3	Extremely chlorotic
9 10	III R_3S_3	None	3.53	24.7 25.6	25.2	Dark green
11 12		0.025 *M* K_2SO_4	3.56	23.4 23.0	23.2	Dark green
13 14		0.025 *M* H_2SO_4	3.11	27.3 25.0	26.2	Very dark green
15 16		0.05 *M* KOH	4.84	15.4 14.6	15.2	Very chlorotic
17 18	Special 1–12	None	4.02	26.0 22.6	24.3	Chlorotic
19 20		0.025 *M* K_2SO_4	4.06	22.6 30.7	26.2	Chlorotic
21 22		0.025 *M* H_2SO_4	3.06	41.3 37.4	39.9	Very dark green
23 24		0.05 *M* KOH	6.27	15.2 17.4	16.3	Very chlorotic
25 26	Special 1–24	None	4.33	21.2 26.2	23.7	Very chlorotic
27 28		0.025 *M* K_2SO_4	4.37	20.0 18.2	19.1	Very chlorotic
29 30		0.025 *M* H_2SO_4	3.14	49.0 48.8	48.9	Very dark green
31 32		0.05 *M* KOH	7.00	17.0 19.1	18.1	Very chlorotic

GROWTH

The curves given in figure 1 show the relation between the pH values and the green weight of tops for wheat plants growing in sand cultures and supplied with the different nutrient solutions. In considering the rôle of the H-ion concentration and its relation to growth, the relative merit of the basic nutrient solutions must be taken into consideration. An examination of the curves of figure 1 shows that solutions "Special 1-12 and 1-24" when adjusted to the proper H-ion concentration are distinctly superior to I R_3S_3 and III R_3S_3. It is impossible, therefore, to plot all pH values and all green weights of tops against each other regardless of the basic nutrient solutions used. It will be

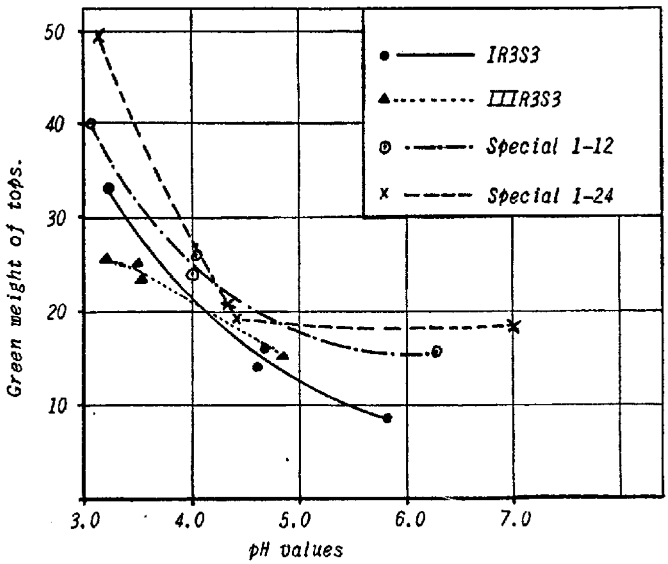

FIG. 1. GREEN WEIGHTS PLOTTED AGAINST pH VALUES

seen from table 2 that all solutions, except III R_3S_3 were greatly improved with respect to the green weight of tops when 0.025 *M* sulfuric acid was added at the rate of 20 cc. per liter of nutrient solution. The addition of the same amount of 0.05 *M* potassium hydroxide depressed the growth to a marked extent. Since an equivalent amount of 0.025 *M* potassium sulfate produced no noticeable effect, all significant variations in growth can be attributed to the differences in the H-ion concentration. The addition of sulfuric acid to III R_3S_3 did not increase the growth rate. This solution is not only quite acid to begin with, but as will be pointed out later, is also highly buffered. It is not surprising, therefore, that this solution behaves somewhat differently

from the other three solutions under consideration. From the curves of figure 1 it will be seen that in every case the highest green weight of tops was secured from the solutions whose pH values were less than 4.

Hoagland (2) reports that solutions having a pH value of 5.0 were not injurious to barley plants. Our results also favor a much higher H-ion concentration for optimum growth of wheat plants than does the work of Duggar (1) and that of Salter and McIlvaine (6). It should be mentioned, however, that these workers employed solution cultures, whereas our plants were grown in sand cultures.

CHLOROSIS

Regardless of the basic nutrient solution used, the H-ion concentration was closely correlated with the presence or the absence of chlorosis in the plants of our cultures. The chlorotic condition varied from a slight tendency toward a light green color to a practically complete absence of chlorophyll.

The seed was placed on the germinator October 23 and 5 days later the seedlings were transferred to the culture pots. About November 5 slight chlorosis was evident in some of the cultures but it was impossible to correlate this chlorotic condition with the H-ion concentration until about 10 days later. From November 15 until the plants were harvested, all solutions having pH values of 3.06 to 3.56 produced normal green plants, while those having pH values of 4.02 to 7.00 produced chlorotic plants.

The physiological rôle of the H-ion concentration is not understood. The H-ion concentration may, of course, have a direct influence upon metabolism, or it may affect the cell permeability or it may have a direct effect upon the condition of the nutrient solution itself. The appearance of the etiolated plants together with the fact that no iron was employed in these culture solutions, except that contained in the sand, at once suggested iron chlorosis.

There are a number of different forms of chlorosis, the most common of which is usually attributed to the lack of available iron. In the field this condition is supposed to be caused by a lack of available iron due to the presence of excessive amounts of lime or other bases. The reaction of the soil solution may render the iron insoluble and thus cause it to become unavailable to the plant in sufficient amounts, or it may have a direct effect upon the metabolism of the plant itself, thus rendering immobile such iron as may actually get into the plant. Although we have no direct positive evidence that the chlorotic condition of our culture plants was due to faulty iron absorption or metabolism, a number of very interesting facts may be mentioned in connection with the iron situation.

In common with many other workers employing sand cultures, we have always regarded the iron contained in the sand as being available in sufficiently large amounts to supply the needs of our plants. There is a strong possibility that this is not true for all nutrient solutions. In a recent publication (5) Patten and Mains have shown that when sodium hydroxide or ammonium

hydroxide is added to a dilute solution of ferric chloride, ferric hydrate ($Fe(OH)_3$ is precipitated, if the resulting mixture has a pH value higher than 3.5. In their solutions a faint cloudiness was apparent at pH = 3.5, which increased to a fine precipitate at pH = 5.5, becoming very heavy at pH = 6.0. Nutrient solutions which were in contact with our growing plants from December 6 to 9 were tested for iron by means of potassium sulfocyanate, with the result that only cultures 13, 14, 21, 22, 29 and 30 gave a positive reaction for iron. It will be noticed that all of these nutrient solutions have a pH value between 3.06 and 3.14.

From December 14 until the time of harvesting, cultures 28 and 32 were given ferric nitrate ($Fe(NO_3)_3$) at each feeding at the rate of 10 parts of iron per million of the culture solution. Culture 26 was given the regular amounts (20 cc.) of 0.025 *M* sulfuric acid. The added iron produced no apparent effect whatever. It will be noticed however that the cultures to which iron was added had pH values of 4.37 and 7.00. These pH values are such that one would expect that the iron would be thrown out of solution as ferric hydrate ($Fe(OH)_3$) and thus made unavailable to the plants. Culture 26, to which 20 cc. of 0.025 *M* sulfuric acid was added soon began to show a decided improvement over no. 25, its check, to which no additions of acid were made. At the time of harvest, culture 26 was normal in color while no. 25 retained its chlorotic condition to the end of the growth period. This again serves to emphasize the importance of the H-ion concentration, although it does not throw any light upon the effect of the reaction on the physiological functioning of the solution.

THE BUFFER ACTION OF THE NUTRIENT SOLUTIONS

In order to study the buffer properties of the nutrient solutions, the hydrogen-ion concentrations of the original basic solutions were determined before and after the 10-cc. and 20-cc. additions of the modifying solutions were made. All of the plant cultures, however, were grown in the original solutions and in solutions modified by the additions of 20-cc. portions of potassium sulfate, sulfuric acid or potassium hydroxide per liter of the nutrient solution.

In table 3 are recorded the effects of these additions upon the hydrogen-ion concentration of the nutrient solution. The buffer properties of the four basic solutions are brought out in the titration curves of figure 2. These curves, within the ranges covered, may be made use of in the following ways:

1. To determine the pH value at which a given solution is the most highly buffered, and conversely, the reaction at which a solution is most easily subject to changes in pH values.

2. To determine the amount of sulfuric acid or of potassium hydroxide that must be added to the basic solutions to secure any desired pH value. This type of information concerning nutrient solutions is very important for several reasons. It makes it possible systematically to prepare solutions of desired H-ion concentrations and to predict something as to the permanency of the

pH value of a given solution when exposed to plants for any length of time, since the highly buffered solutions are more resistant to change than those that exhibit but little buffer action. It is, of course, desirable to use nutrient

TABLE 3

The effect upon the hydrogen-ion concentration of the additions of different amounts of acid and alkali to basic culture solutions

BASIC SOLUTIONS	ADDITIONS TO BASIC SOLUTIONS	HYDROGEN-ION CONCENTRATIONS	
		After additions of 10 cc.	After additions of 20 cc.
		pH	*pH*
I R_3S_3	None	4.60	4.60
	0.025 *M* K_2SO_4	4.63	4.68
	0.025 *M* H_2SO_4	3.60	3.26
	0.05 *M* KOH	5.56	5.83
III R_3S_3	None	3.53	3.53
	0.025 *M* K_2SO_4	3.55	3.56
	0.025 *M* H_2SO_4	3.28	3.11
	0.05 *M* KOH	3.94	4.84
Special 1–12	None	4.02	4.02
	0.025 *M* K_2SO_4	4.06	4.06
	0.025 *M* H_2SO_4	3.32	3.06
	0.05 *M* KOH	5.88	6.27
Special 1–24	None	4.33	4.33
	0.025 *M* K_2SO_4	4.37	4.37
	0.025 *M* H_2SO_4	3.42	3.14
	0.05 *M* KOH	6.42	7.00

TABLE 4

Magnitude of the change in pH values, produced by the addition of sulfuric acid and of potassium hydroxide to basic solutions

BASIC NUTRIENT SOLUTION	pH VALUES OF ORIGINAL SOLUTIONS	CHANGE IN pH VALUES ON ADDITION OF			
		0.025 *M* H_2SO_4		0.05 *M* KOH	
		10 cc.	20 cc.	10 cc.	20 cc.
I R_3S_3	4.60	1.00	1.34	0.96	1.23
III R_3S_3	3.53	0.25	0.42	0.41	1.31
Special 1–12	4.02	0.70	0.96	1.86	2.25
Special 1–24	4.33	0.91	1.19	2.09	2.67

solutions whose reaction remains reasonably constant and thus avoid too frequent renewals of the solutions.

The data of table 3 are summarized in table 4 to show the magnitude of the changes in the pH values that are produced by certain additions to the basic nutrient solutions.

This table, in a general way, brings out the relative ease with which hydrogen-ion concentration of any solution may be changed by the addition of various increments of sulfuric acid or of potassium hydroxide.

Conversely, the same data will be found useful in the study of changes in the pH values of nutrient solutions after contact with plant roots. This information, of course, can be obtained directly from the titration curves. These suggestions are in agreement with the work of Salter and McIlvaine (6).

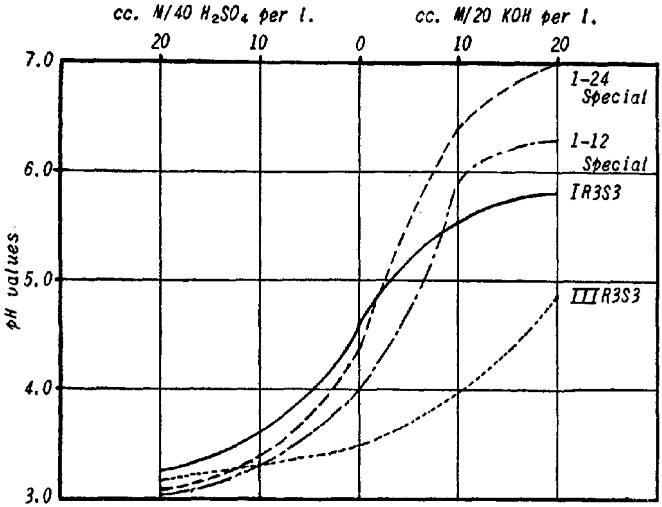

FIG. 2. TITRATION CURVES FOR BASIC SOLUTIONS USED[2]

SUMMARY

1. Wheat plants were grown for 2 months in four different basic nutrient solutions, each of which was modified in such a way as to have three distinctly different pH values without materially altering the solutions with respect to the concentration of the six essential ions.

2. The H-ion concentration not only exerted a very marked influence upon the growth rate but also was an important factor in the control of chlorosis.

3. Although the exact rôle of the H-ion concentration is not understood, the appearance of the chlorotic plants and some indirect evidence would indicate that our plants grown in solutions having pH values ranging from 4.02 to 7.0 were suffering from the lack of available iron or from faulty metabolism resulting from the immobility of the iron within the plants.

4. The data presented emphasize the importance of the proper control of the H-ion concentration in all work of this kind.

[2] The terms M/40 H_2SO_4 and M/20 KOH that appear in figure 2 are equivalent to the expressions 0.025 M H_2SO_4 and 0.05 M KOH, respectively, as given in the text.

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EFFECTS UPON THE GROWTH OF POTATOES, CORN AND BEANS RESULTING FROM THE ADDITION OF BORAX TO THE FERTILIZER USED

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In 1876 Peligot (14) grew beans in porous pots, the soil of which was moistened with a 0.2 per cent solution of either boric acid, potassium borate or sodium borate. He reported that the plants were killed in all cases. Nakamaura (13) states that borax at the rate of 50 mgm., and in some cases 10 mgm. per kilogram of soil, was harmful to plants in pot cultures. Within stated limits Agulhon (1) found that boric acid caused a stimulation of the growth of wheat in water cultures. He also maintains (2) that the seeds of corn grown and matured in soil containing boric acid, when planted again in such soil, produced plants which were more resistant to boric acid and gave larger yields than plants from seeds grown under normal conditions. Working with water cultures, Brenchly (3) found that boron was one of the inorganic substances which may both poison plants and cause a stimulation within certain limits—the stimulation differing for different plants. Lipman (9) found that a commercial limestone containing boron, besides depressing plant growth also depressed the ammonifying power of the micro-flora of the soil. Cook (5) studied the absorption and distribution of boron in certain cereals, legumes and garden vegetables when borax and calcined colmanite had been added, as larvicides, to the manure used as fertilizer for these plants. This work was continued by Cook and Wilson (7), who paid particular attention to the effects of the presence of boron upon the plants themselves. The same writers (6) also have reported upon the effect of three annual applications of boron on wheat. At the rates applied, the addition of borax and colmanite to manure used to fertilize peach trees seemed to have a stimulating effect.

[1] Dr. Neller introduced the system of soil moisture control and had sole charge of the experimental work recorded herein. The last named author is responsible only for the original, detailed plans and for assistance in the final revision of the manuscript.—W. J. M.

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No injury was observed with some of the other varieties of plants tested, while with others visible injury or reduction of yields or both occurred, and this amount of injury increased as the amount of boron added was increased. From the results of these experiments it is evident that a considerable variety of agricultural crops can be grown in comparative safety on soils to which relatively large amounts of borax have been applied, provided this borax is first thoroughly mixed with a considerable quantity of stable manure. A number of different factors are involved, however, for the authors state (7, p. 470) that:

> The absorption of boron by plants varies with the variety of plant, the solubility of the boron compound, the quantity of the boron compound added to the soil, the time elapsing after the compound is mixed with the soil before planting, the amount of rainfall, etc., and finally with the character of the soil to which the boron compound is added.

Recent field observations in different parts of the United States, which have been reported by Proulx and his associates (15), Conner (4), Morse (12), and Schreiner, Brown, Skinner and Shapovalov (16), have shown that definite and often severe types of crop injury are frequently associated with previous applications of fertilizers containing boron in water-soluble form. This led one of the writers (12) to conduct a series of greenhouse experiments in the fall and winter of 1919–20, mostly with potatoes, in which the effects of commercial fertilizers containing borax in various amounts were compared with those obtained from a similar, but not otherwise identical, fertilizer which was borax-free.

In these experiments definite and in some instances severe injury was obtained where fertilizers containing borax were used for potatoes and beans grown in pots in the greenhouse. No injury to the same crops resulted from the use of a borax-free fertilizer otherwise of similar composition. It was recognized that these preliminary experiments were deficient in certain respects. Possibly the most fundamental objection that could be raised to them was that while they showed quite clearly that the injury occurred only where fertilizers containing borax were applied, they provided no conclusive data to prove that borax was the sole toxic agent involved. It was planned to repeat and amplify these experiments as far as the limitations of greenhouse space at the Maine Station would permit. While the work was in progress Director Woods of Maine, at a meeting of the directors of the Maine, New Hampshire, Vermont, Massachusetts, Rhode Island, Connecticut, New York and New Jersey stations, made a report of the results already obtained. In the discussion which followed it developed that the executives of these various stations were all interested in making a study of the toxic effect of borax in commercial fertilizers. From the standpoint of both efficiency and economy it was evident that such a study could be carried out best as a single joint project, each station bearing its proportionate part of the expense involved. Fortunately, a greenhouse well adapted to the purpose was avail-

able in Vermont and Director Hills' offer to place this at the disposal of the cooperating stations was accepted. The director and pathologist of the Maine station were requested to assemble the necessary fertilizer materials and to prepare the detailed plans for the experimental work. Director Lipman of New Jersey was given authority to select a suitable person to carry on the greenhouse work in Vermont. These details are mentioned in order to make it clear that the work here reported was undertaken and carried out under the joint auspices of eight different institutions in as many different states. In this respect it is believed that it establishes a new record for cooperation among experiment stations.

GENERAL SCOPE OF THE EXPERIMENTS

The chief purpose of the experiments herein described was to determine whether the injuries previously observed both in the field and in the greenhouse were due alone to the borax present in the fertilizers applied and, if so, what is the maximum amount of borax that can be applied per acre to land on which important food crops are to be grown, without causing this injury. Potatoes, corn and beans were selected as representatives of three different types of such crops. The plan outlined provided that the soil in a pot containing borax, for example, should differ only in this one respect in its fertilizing treatment from a pot of the same soil containing none of this substance. Hence, as is pointed out below, all the fertilizer used consisted of a single basal mixture prepared from materials of known composition and borax-free. As will be seen on reference to the detailed account of the work, other important questions were considered also. Attempts were made to determine the effect of distributing the fertilizer above and below the seed or seed-piece, both in drills and mixed with the soil.

Recently several persons have reported a neutralizing effect of certain calcium salts toward inorganic plant poisons. McHargue (11) found that the addition of small amounts of calcium carbonate checked the poisonous effects of barium and strontium salts and caused them to be stimulating at certain concentrations. Truog and Sykora (17) tested the influence of calcium carbonate and kaolin on the inhibition of the toxic effects of copper sulfate, copper nitrate, vanillin, sodium arsenite and quinidine carbonate and obtained positive results. The effect of lime upon the sodium chloride tolerance of wheat seedlings was found by Le Clerc and Breazeale (10) to be quite marked.

The work of Cook and Wilson (7) indicates that stable manure might in some way neutralize or prevent the toxic action of boron upon plant growth. For this reason it seemed desirable to attempt to determine whether some substance could be added to fertilizers containing borax, which would neutralize its toxic properties either wholly or in part. Ground limestone, hydrated lime, gypsum and manure were the materials employed for this purpose in the experiments under consideration. The amounts and the methods of use are discussed in the sections devoted to the different crops tested.

EXPERIMENTAL PROCEDURE

The soil available during the winter of 1920 when this work was started, had been composted with lime and a medium amount of manure for over a year. It was allowed to become nearly air-dry and was then passed through a ¼-inch mesh sieve. This removed some of the organic matter, the content of which was further reduced by adding sand equal to about one-third of the volume of the soil. As used the soil resembled a sandy clay loam and had a maximum water capacity of 37.5 per cent by the Hilgard method (8). It contained 4.22 per cent of volatile matter and had a lime requirement, or absorption coefficient, of 320 pounds per acre (2,000,000 pounds of soil).[2] The pots employed consisted of solid glazed jars. Two sizes were used, of diameter 8¼ and 5½ inches and of depth 8½ and 7 inches, respectively. The use of solid glazed jars prevented any possible accumulation of borax or fertilizing constituents such as may occur in the walls of porous pots as a consequence of the constant evaporation of the soil water from their outer surfaces. Glazed pots also permit a more definite control of the amount and distribution of the soil moisture. Their use provides soil conditions more nearly like those existing in the field.

The fertilizer which was used was made from borax-free commercial salts —sodium nitrate, acid phosphate and potassium sulfate—containing, respectively, 18.96 per cent of ammonia, available phosphorous equivalent to 18.00 per cent of phosphoric oxide, and 49.68 per cent of potassium oxide. This was applied in 4–8–6 proportions on the basis of 2000 pounds of mixed fertilizer per acre for the potatoes and at the rate of 500 pounds per acre for corn and beans. The sodium nitrate was ground in a mill to insure more thorough mixing with the phosphate and sulfate.

The amount used per pot was computed from the amount which would be applied in the field in drills 33 inches apart, the length of drill corresponding to the diameter of the pot. Thus 29.98 gm. of the mixed fertilizer per pot was used for the potatoes and 5.15 gm. for the beans and corn. The required portions were weighed separately into small bottles.

Definite amounts of borax were then added to the borax-free fertilizer. The "20 Mule Team" commercial brand which contained 67.2 per cent of anhydrous borax was added at the rates shown in the table on the following page.

The borax was not mixed with the ulbk fertilizer but was weighed for each pot, added to the above-mentioned bottles and mixed with the fertilizer by shaking. No borax was added to the fertilizer which was used in the check pots and neither borax nor fertilizer was added to those designated as controls. Unless otherwise stated all tests were made in triplicate; that is, there were 3 pots of potatoes, corn or beans for each treatment in each series listed.

[2] The writers wish to acknowledge their indebtedness to J. M. Bartlett, chemist of the Maine station, for analyses of the materials used for the fertilizers and to C. H. Jones, chemist of the Vermont station, for similar assistance and more particularly for making tests for borax in leaves and other plant parts.

RATE PER ACRE OF ANHYDROUS BORAX	WEIGHT OF THE "20 MULE TEAM" BRAND APPLIED	
	Per 5½-inch pot	Per 8-inch pot
pounds	*gm.*	*gm.*
1	0.0193	0.2806
2	0.0386	0.5613
4	0.0772	
5	0.0965	0.1403
6	0.1158	
8	0.1544	
10	0.1930	0.2806
20	0.3860	0.5613

The Green Mountain variety of potatoes was used for the tests. Unblemished tubers of uniform size were selected and sliced lengthwise, each seed-piece weighing from 4 to 6 ounces. Eleven and one-half pounds of soil were added to each 8-inch pot and one seed-piece was planted, with cut portion down, after which 5 pounds of soil were added, making the seed-piece about 3 inches below the surface of the soil. In those series in which the fertilizer was applied in drills it was shaken out of the bottles through a perforated aluminum cap and evenly distributed over a strip about 3 inches wide across the diameter of the pot. When applied in drills a layer of soil about ¼ inch thick was spread between it and the seed-piece. When broadcasted it was mixed with a given quantity of soil. This could be done quite thoroughly as the soil was nearly air-dry.

The Bush Horticultural variety of beans and the Leaming dent variety of corn were used. These lots of seeds gave germination tests of 100 and 94 per cent, respectively. Five seeds per pot were planted at a depth of 1 inch and seedlings appearing after the first three were removed. Five and one-half pounds of soil was first added to each 5½-inch pot, the seeds planted with uniform spacing over a strip 3 inches wide and then covered with ¾ pound of soil. The fertilizer was applied in the same manner as described for potatoes, with a thin layer of soil between it and the seed.

In order that the soil might be watered from the bottom as well as from the top a cardboard tube about 1½ inches in diameter was set against the vertical wall of each pot. After dipping these tubes in hot paraffin they became water-tight and no evaporation took place from them. About two-thirds of the initial water, added to the soil, was introduced through the tubes from measured flasks. The remainder was added with a sprinkling can and the final weight of the pots recorded. Except where specifically stated 19.2 per cent of water was maintained in the soil of all pots, this being 50 per cent of its water-holding capacity, previously determined.

Water was added to restore the amount lost as shown by a decrease in the weight of the pots. After the plants became fairly large this was done each day. An estimated allowance was made for the increase in pot weights due to the green matter of the plants. This estimate was found to be fairly close

to the green weights of the plants at the time of harvesting. After harvesting the plants, three of the bean and two of the potato pots, selected at random, were found to have soil moisture contents of 19.1, 21.3, 18.9, 15.9 and 16.7 per cent, respectively. This shows that the variation from the desired 19.2 per cent was not excessive. The moisture appeared to be evenly distributed throughout the soil in each pot.

During the germination period and until the plants became uneven in size, one-half of the water was added to the bottom of the soil through the paraffined tubes and one-half was added to the surface of the soil. After the pots began to require very unequal amounts of water it was all added to the surface soil.

From the time of planting, February 2, to that of harvesting, April 22, the nightly and daily temperatures of the greenhouse averaged 58.0° and 68.5°, respectively. The average relative humidity was 57.3 per cent and the hours of sunshine were 42.8 per cent of the total possible amounts.

After plants appeared above ground, detailed notes on the growth and appearance of each were made every 7 to 10 days. The photographs of representative pots and the crop-weight averages are of plants which grew in the one that most nearly represented the average of the triplicately treated pots.

EFFECT OF BORAX UPON THE GROWTH OF POTATOES

For potatoes the borax applications per pot were at the rate of 2, 5, 10 and 20 pounds of anhydrous borax per acre. The applications of fertilizer, with and without borax, were made in drills below the seed-piece (series 1); in drills above the seed-piece (series 2); mixed with the second 3 inches of soil (series 3) and mixed with the first, or top 3 inches of soil (series 4). Series 5 received fertilizer and borax at the rate of 5 pounds per acre in drills below the seed-piece together with additions of either ground limestone, hydrated lime, gypsum or manure at the rates of 2,000, 1,000, 1,000 and 20,000 pounds per acre, respectively. Series 6 was similar to series 5 except that borax was used at the rate of 10 pounds per acre. The water content of the soil was maintained in the manner given under the section on experimental procedure.

Foliage abnormalities and injuries[3]

Plants were showing in nearly all of the pots a month after the seed-pieces had been planted. At that time, March 4, certain abnormalities were noted in most of the plants growing in pots to which borax had been added at the rates of 10 and 20 pounds per acre. In some cases the young plants appeared

[3] For a description of the types of injury to potatoes as observed in the field and in the greenhouse by one of the writers, where commercial fertilizers containing borax were used, the reader is referred to Bulletin 288 of the Maine Agricultural Experiment Station (12, p. 95–101, 107–118).

to be stunted, dwarfed and generally of a lighter green color than the checks. In other cases they looked normal except for an upward curling of the terminal leaflets of some of the lower leaves, accompanied by death of the leaflet margins especially at the tips. A week later those plants which were light green and stunted had become more nearly normal in color. They always remained in a stunted condition, however, and began to show a type of injury which appeared to be most typical for the potato plant.

This injury appeared on many of the plants which at first seemed perfectly healthy, and consisted in its first stages of death of the margins of the leaves, especially at the tips of the margins of the terminal leaflets of the oldest leaves. This was soon followed by a change in color of the entire leaflet to a greenish or golden yellow which then spread throughout the entire leaf as may be noted in pot 57 (pl. 1, fig. 1). If badly affected, the leaf died and fell off. Where injury was excessive this process moved upward until most of the leaves had fallen off, while all of them were badly injured, as may be noted in pot 60 (pl. 1, fig. 3) to which borax had been added at the rate of 20 pounds per acre in the upper 3 inches of soil. When the upper leaves became affected, however, they did not change to a golden color, and often dead areas, similar to the marginal injury, appeared in the interior, surrounded by somewhat less injured tissue. Plate 1, figure 2, from left to right, shows a normal leaf, one slightly injured, and one with killed marginal tissue and golden yellow interiors, the final death stage of which would be like the two leaves at the right. The third and fourth leaves from the right are from the upper part of a badly affected plant like that of pot 60 (pl. 1, fig. 3). Pot 56 (pl. 1, fig. 1) received borax at the rate of 10 pounds per acre mixed with the upper 3 inches of soil and shows plants (photographed March 29) which have been stunted as compared with the normal plants in pot 73. Pot 13 of plate 1, figure 3, which received borax at the rate of 20 pounds per acre (photographed April 22), shows an even more pronounced case of stunting as well as leaf injury caused by borax. The plants of pot 25, which received half as much borax as pot 13, grew well and appeared normal at first but later became quite badly affected, as may be noted in the photograph.

Effect of method of application

The photographs reproduced in plates 2 and 3 were taken March 9 and show all of the pots of series 1, 2, 3 and 4, the borax applications from right to left being at the rates of 0, 2, 5, 10 and 20 pounds per acre. At that time the manner of applying the borax-fertilizer mixture was beginning to show a marked effect. At that stage of growth, the applications in drills below the seed-pieces (series 1, pl. 2, fig. 1) were much more toxic than in drills above (series 2, pl. 2, fig. 2). In the same way plate 3 shows that the applications to the 3 inches of soil below the seed-pieces (series 3, fig. 1) caused more retardation and injury than those to the upper 3 inches of soil (series 4, fig. 2).

Moreover, of the applications below the seed-pieces, those in drills caused more toxicity than those which were broadcasted. It was also apparent from the checks that the borax-free fertilizer did not give as good results when applied below as when applied above the seed-pieces, but this difference disappeared later. As may be noted from these photographs, a number of the plants of the 10 and 20-pound borax lots in series 2 and 4 appear nearly normal. Later, however, they developed the characteristic lower leaf injury.

In general, there was a pronounced increase in injury in passing from the 5 to 10-pound borax pots. The plants in the two pots showing in the foreground of the photograph of series 1 were the only ones of the checks, the 2-pound and the 5-pound borax lots which did not develop well.

Horizontal views of representative pots of the 4 series were photographed on March 29 and are shown in plate 4 and in figure 1 of plate 5. A decided decrease in the size of the plants of the 10- and 20-pound borax pots is evident where the fertilizer was applied in drills below the seed-pieces (pl. 4, fig. 2) as well as when broadcasted in the second 3 inches of soil (pl. 4, fig. 3). The plants are more even in size in the pots in which the fertilizer was applied above, either in drills (pl. 4, fig. 1) or when mixed with the upper 3 inches of soil (pl. 5, fig. 1). This last-mentioned figure shows that the lower leaves had become badly affected where borax had been applied at the rates of 10 and 20 pounds per acre. The 5-pound borax lot also was slightly affected. At first this lower leaf injury was least marked in the series in which the fertilizer had been applied in drills above the seed-pieces, but it developed later to a considerable extent.

Relative amounts of leaf injury at the time of harvesting

During the two weeks previous to the harvesting the plants did not grow to any appreciable extent, nor was there much increase in borax injury. At the time of harvesting practically no lower leaf injury was noted on the plants of the 2-pound borax lots, and only a slight amount in those of the 5-pound lots. For a given series there was not much difference, on the average, in the amount of injury caused by the 10 and 20-pound applications. A general stunting of the plants was most marked where the fertilizer had been applied in drills below (series 1). Series 4, in which the fertilizer had been mixed with the upper 3 inches of soil, showed the most leaf injury and some of the plants, as in pot 60 (pl. 1, fig. 3), had lost most of their leaves, while those remaining were dry, crisp and practically dead.

Foliage and tuber weights

Tables 1 and 2 give the dry weights of the tops for the triplicate treatments and the green weights of the tubers which developed. These figures may be more readily compared in table 3 which shows the weights, relative to the

checks, which are given a value of 100. These values are based on the average of weights given in tables 1 and 2.

It is well known that individual seed-pieces give very uneven yields, even when obtained from the same hill of potatoes. Consequently, irregularities may be expected in these tests since the seed-pieces were halves of potatoes

TABLE 1

The dry weights of foliage and green weights of tubers of potato plants grown in pots to which fertilizer and definite amounts of borax were added

	CHECK, NO BORAX		2 POUNDS OF BORAX		5 POUNDS OF BORAX		10 POUNDS OF BORAX		20 POUNDS OF BORAX	
	Tops	Tubers	Tops	Tubers	Tops	Tubers	Tops	Tubers	Tops	Tubers
Series 1. Borax-fertilizer mixtures in drills below the seed-pieces										
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Pot 1	15.68	415.0	21.89	436.0	15.04	241.0	5.80	94.5	*	85.0
Pot 2	20.90	0	21.14	287.0	12.81	232.0	21.30	222.0	6.43	54.5
Pot 3	5.66	426.0	10.18	17.0	10.44	298.5	1.54	0	7.80	11.5
Average	14.08	280.3	17.70	297.7	12.76	291.5	9.54	105.5	7.11	50.3
Series 2. Borax-fertilizer mixtures in drills above the seed-pieces										
Pot 1	18.53	455.0	16.03	393.0	23.42	382.0	9.60	276.0	11.57	173.5
Pot 2	18.39	581.0	14.86	272.0	11.55	170.0	18.84	307.0	11.59	280.0
Pot 3	9.63	263.0	13.75	426.0	11.63	322.0	17.30	158.0	10.79	270.0
Average	15.52	433.0	14.88	397.0	15.53	291.3	15.24	247.0	11.44	241.2
Series 3. Borax-fertilizer mixtures in the second 3 inches of soil										
Pot 1	17.04	185.5	9.28	192.0	16.63	299.0	3.14	390.0	2.66	41.5
Pot 2	19.50	396.0	7.92	348.0	11.19	406.0	18.72	326.0	5.58	81.5
Pot 3	12.53	233.0	12.64	312.0	18.12	313.0	22.70	253.5	6.24	80.0
Average	16.36	271.5	9.95	284.0	14.65	339.3	14.89	323.2	4.83	67.5
Series 4. Borax-fertilizer mixtures in the first 3 inches of soil										
Pot 1	18.30	523.0	14.45	238.5	11.68	296.0	10.75	185.5	11.81	301.0
Pot 2	14.69	251.5	14.88	360.0	18.34	316.0	6.38	64.5	5.88	155.5
Pot 3	13.88	365.5	15.46	291.5	14.56	358.0	5.91	186.0	5.66	101.0
Average	15.62	380.0	14.93	296.7	14.86	323.3	7.68	145.0	7.78	185.0

* Used for chemical determinations.

selected from a large number to obtain tubers which were unblemished and even in size. It is evident, nevertheless, that there is a considerable decrease in tuber weight where borax was added at the rate of 10 pounds per acre, except in series 3 (table 3). Where the borax applications were at the rate of 20 pounds per acre there was a marked decrease in every case, especially where the applications were below the seed-pieces (series 1 and 3), in which

the yield averaged 22 per cent of that given by the checks. Where the applications were above the seed-pieces (series 2 and 4) the corresponding average yield was 53 per cent of that given by the checks. The dry weights of tops are more variable but do not show any marked decrease in the 2 and 5-pound borax lots. Considerably decreased weights are evident in some of the 10-pound and in all of the 20-pound lots. In the latter case the weights are lowest where the borax-fertilizer mixture was applied below the seed-pieces. In general, foliage and tuber weights correlate with the above described borax injury, which was based upon notes taken while the plants were growing.

TABLE 2

The dry weight of tops and green weight of tubers of potato plants grown in pots to which ground limestone, hydrated lime, gypsum or manure had been added in addition to the borax-fertilizer applications

	GROUND LIMESTONE				HYDRATED LIME				GYPSUM				MANURE	
	In drills		In upper 3 inches		In drills		In upper 3 inches		In drills		In upper 3 inches		In upper 3 inches	
	Tops	Tubers	Tops	Tubers	Tops	Tubers	Tops	Tubers	Tops	Tubers	Tops	Tubers	Tops	Tubers
Series 5. Fertilizer and 5 pounds per acre of borax in drills below the seed-piece														
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Pot 1	18.07	297.5	7.51	66.5	9.68	230.0	8.21	180.0	18.78	526.0	lost	187.0	15.36	245.0
Pot 2	18.55	342.0	23.12	344.0	13.99	379.5	8.54	131.0	0	0	5.76	106.0	13.50	223.0
Pot 3	17.86	583.0	19.50	248.0	9.47	0	12.76	210.0	11.18	215.5	7.80	83.5	14.11	247.5
Average ...	18.16	407.5	16.63	219.5	11.05	203.2	9.84	174.0	9.99	247.2	6.78	125.5	14.32	238.5
Series 6. Fertilizer and 10 pounds per acre of borax in drills below the seed-piece														
Pot 1	2.08	5.0	4.46	23.0	17.97	289.0	13.13	271.5						
Pot 2	21.96	433.0	9.79	62.5	18.77	371.5	13.41	142.0						
Pot 3	12.38	361.0	16.87	271.0	17.13	298.0	13.57	296.0						
Average ...	12.14	266.3	7.34	118.8	17.98	286.2	13.37	236.5						

Effect of borax upon the roots of potatoes

The root systems and yields of representative pots from the four series, discussed above, are shown in figure 3 of plate 5 and in the figures of plate 6. It may be seen that the root systems and tuber yields are smaller from the 10 and 20-pound borax applications, with the exception of the 10-pound applications of series 3, pot 41 (pl. 6, fig. 3). Table 3 also shows that the average weights for that treatment were much like those of the checks.

The browned tips of decayed roots were evident in the 10 and 20-pound lots of some of the pots of series 1 and 4. In cases of severe injury as in pot 60 (pl. 1, fig. 3) the stems both above and below the surface of the soil were soft and tough, with occasional brown-colored lesions on the underground portions. In general, however, injuries due to borax were not as much in evidence on the underground parts as on the aerial portions of the potato plants grown in pots in the greenhouse.

Effects of using lime, gypsum and manure with borax-fertilizer mixtures

Table 4 gives the weight values, relative to the checks, resulting from the addition of either finely ground limestone, hydrated lime, gypsum or manure at the rate of 2,000, 1,000, 1,000 and 20,000 pounds per acre, respectively. The lime applications were with borax-fertilizer mixtures applied in drills below the seed-pieces. Thus the 5-pound-per-acre borax pots of series 1 constitute the checks for series 5 (table 4), while the 10-pound borax pots of series 1 are the checks for series 6. A photograph (pl. 5, fig. 2) taken 25 days before the plants were harvested shows that the toxic effect of borax added at the rate of 10 pounds per acre (pot 10) was considerably neutralized by mixing it either with hydrated lime (pot 88) or with a finely ground limestone (pot 84). Pot 62 which received ground limestone mixed with fertilizer and borax at the rate of 5 pounds per acre looked somewhat better than the corresponding unlimed pot no. 9. No benefit was observed where gypsum (pot 69) was applied; nor did the manure cause much effect other than a more sustained growth rate during the one or two weeks before the time of harvesting.

TABLE 3

Weights of tubers and of tops relative to the checks, which are given a value of 100; based on the averages given in table 1

BORAX APPLIED PER ACRE	IN DRILLS BELOW (SERIES 1)		IN DRILLS ABOVE (SERIES 2)		IN SECOND 3 INCHES (SERIES 3)		IN FIRST 3 INCHES (SERIES 4)	
	Tubers	Tops	Tubers	Tops	Tubers	Tops	Tubers	Tops
lbs.								
0	100	100	100	100	100	100	100	100
2	106	126	92	96	105	61	78	96
5	104	90	67	100	125	90	85	95
10	38	68	57	98	119	91	38	49
20	18	51	56	73	25	29	49	50

The relative dry weights (table 4) are in accord with the notes taken during the growing season and show that, in general, lime neutralized some of the toxicity of borax when the latter was applied at the rate of 10 pounds per acre; while neither lime, gypsum nor manure exerted any appreciable effect when used with borax applied at the rate of 5 pounds per acre. However, borax at the rate of 5 pounds per acre was practically non-toxic and hence the possible effect of gypsum and of manure was not conclusively tested in this experiment.

Results obtained with a commercial fertilizer containing borax[4]

A few pots were fertilized with a commercial fertilizer which contained somewhat less than 1 per cent of borax. Where the applications were at

[4] This is the same fertilizer as is listed as "Station No. 5549" on page 105 of Bulletin 288 of the Maine Agricultural Experiment Station (12).

the rates of 2000 and 1000 pounds per acre the same types of injury were observed as developed in the plants of the pots to which borax was added. The number of pots containing this commercial fertilizer was not sufficient, however, to make a comparison of yields or extent of injury with the borax-treated pots.

Borax has been reported as present in the injured portions of leaves of plants grown in pots containing fertilizers which carried this material in varying amounts, but not in the leaves of plants grown on a borax-free fertilizer of a similar composition (12). Similar samples were collected from pots containing the commercial fertilizer mentioned above and from pots containing the basal fertilizing mixture used in these experiments, with and without borax. These were tested by C. H. Jones. Borax was found in the injured leaves from those pots where it was introduced with the fertilizer but not in the leaves from the pots which received the basal mixture alone.

TABLE 4

Weights of tubers and tops relative to the checks which are given a value of 100; based on the averages in table 2

	5 POUNDS OF BORAX PER ACRE (SERIES 5)		10 POUNDS OF BORAX PER ACRE (SERIES 6)	
	Tubers	Tops	Tubers	Tops
Checks—borax-fertilizer mixture only	100	100	100	100
Ground limestone in drills	139	142	25	127
Ground limestone in first 3 inches	75	130	113	77
Hydrated lime in drills	70	87	271	188
Hydrated lime in first 3 inches	60	77	224	140
Gypsum in drills	85	78		
Gypsum in first 3 inches	43	53		
Manure in first 3 inches	82	113		

Summary relative to the effect of borax upon potatoes

The application of 2 pounds of borax per acre caused no injury to potato plants while a few of the plants to which 5 pounds per acre were added exhibited a slight injury to the lower leaves.

The additions of 10 and 20 pounds of borax per acre caused a marked injury and retardation, especially when applied below the seed-pieces.

In general the toxic limit of borax for potatoes under the conditions of the experiment was somewhat above the rate of 5 pounds per acre.

When applied above the seed-pieces there was no noticeable effect on potato foliage when the plants were young; but later they developed excessive leaf injury where borax was present at the rates of 10 or 20 pounds per acre.

Borax-fertilizer mixtures applied below the seed-pieces proved to be the most toxic, particularly when in drills. The plants receiving the heavier applications were stunted from the first and often developed considerable leaf injury also.

The higher borax applications caused considerable injury to root systems and decrease in tuber yields, especially when applied below the seed-piece. Stem injury, both above and below the soil, was most marked where the plants grew normally at first, but were injured later by the borax which had been applied above the seed-piece.

Both finely ground limestone and hydrated lime appeared to neutralize some of the toxicity of borax when applied in drills below the seed-piece at the rate of 10 pounds per acre.

Plants grown in pots fertilized with a commercial fertilizer containing borax developed the same types of injury as occurred where borax-fertilizer mixtures were applied. Boron was found to be present in the affected leaves in both cases.

THE EFFECT OF BORAX UPON THE GROWTH OF CORN

With corn the fertilizer-borax mixtures were applied to the soil in three different ways. Series 1 designates the application in drills below the seed, series 2 in drills above the seed and series 3 mixed with the upper 3 inches of soil. The rates of application of anhydrous borax were 1, 2, 3, 5, 10 and 20 pounds per acre. The plants listed as checks received fertilizer but no borax and those designated as controls had nothing added to the soil. All fertilizer applications were on the basis of 500 pounds per acre. Additional details relative to fertilization, planting, etc., are given under the section on experimental procedure. As stated before, the various treatments were in triplicate.

The seedling stage

Although the corn used for seed gave a germination test of 94 per cent, it did not germinate as well in the soil. Five seeds were planted per pot and the seedlings appearing after the first three were removed. Table 5 shows the total number of those which appeared in the triplicately treated pots of the three series, the checks and the controls. Fifteen seedlings per treatment should have appeared had the germination been perfect. As the table shows it was not perfect either in the checks or in the controls. However, a marked reduction in the number of seedlings which appeared is evident in the 5, 10 and 20-pound-per-acre borax applications in drills below the seed (series 1); and in the 20-pound applications both in drills above and when broadcasted in the upper 3 inches of soil.

By April 2, or 24 days after planting, all except one of the seedlings which appeared in the 5, 10 and 20-pound borax pots of series 1 had died. In series 2 and 3 all seedlings had died in the pots to which fertilizer and borax at the rate of 20 pounds per acre had been added. All of the seedlings in the 10-pound borax treated pots of these two series were stunted and a few of them died.

Some of the seedlings were bleached to a yellowish or whitish tinge from the time of their appearance above the soil. Others looked nearly normal at

first but rapidly turned white and died. Retardation of germination due to borax did not appear to be as marked as with beans but the young seedlings seemed to be more sensitive toward borax. As far as could be observed the death of the seedlings was not due to any influence other than that of borax.

It was readily apparent that the borax-fertilizer mixtures were most toxic when applied in drills below the seed and least toxic when mixed with the upper 3 inches of soil. Moreover, the fertilizer itself appeared to have a depressing effect upon the young corn plants when applied in drills, particularly drills below the seed. This was evident from the uneven stand and irregular growth obtained in series 1 and 2 as compared with the much more even stand and growth obtained in the broadcasted series no. 3. Plants in the control pots (no addition to the soil) grew faster than those in the checks (borax-free fertilizer added), but soon fell behind and developed excessive malnutrition characteristics.

TABLE 5

Effect of different borax-fertilizer mixtures on the germination of corn

POUNDS OF BORAX PER ACRE	IN DRILLS BELOW (SERIES 1)						IN DRILLS ABOVE (SERIES 2)						IN FIRST 3 INCHES (SERIES 3)						NO ADDITION TO SOIL
	0	1	2	5	10	20	0	1	2	5	10	20	0	1	2	5	10	20	
Seedlings per pot 1	3	1	2	1	1	0	2	0	3	4	2	0	2	4	3	3	0	2	5
Seedlings per pot 2	3	2	4	0	0	0	3	3	1	2	2	0	4	5	5	4	5	1	3
Seedlings per pot 3	3	4	5	2	2	2	3	3	4	2	2	1	5	3	5	3	4	2	3
Total per treatment	9	7	11	3	3	2	8	6	8	8	6	1	11	12	13	10	9	5	11

Nature of borax injury to corn

When young corn plants were injured by borax they generally turned almost white and died. The roots of such plants were found to be entirely browned and decayed. Plants which attained a height of 6 inches or more before becoming affected, evidenced borax toxicity in their foliage by a banded bleaching of the chlorophyl of the leaves, especially marked at the leaf margins. The extreme tips of the leaves were often killed but not the margins. The banded bleaching was not as pronounced as a general stunting of the plants which survived the higher borax applications. Very young corn plants appeared to be more sensitive toward borax than young bean seedlings while stalks of corn which obtained a normal start appeared to be considerably less sensitive than bean plants in the presence of the same amounts of borax. This comparison could be made, as the corn and beans were planted at the same time under the same experimental conditions. However, the uneven stand in all but series 3 made the tests with corn less conclusive than those with beans.

Effect upon the roots

Figure 2, plate 7, shows the roots of plants from representative pots of series 2 in which the fertilizer was applied in drills above the seeds. The roots of the 1-pound borax pot no. 102 are much like those of the check pot no. 99 while the 2-pound borax application, pot 103, caused a bushy development of fine roots. Besides the reduction in root systems, where larger amounts of borax were applied, the browned ends of killed roots were observed as is shown in the 10-pound borax pot no. 111.

TABLE 6

Dry weights of corn foliage grown in pots to which fertilizer and definite amounts of borax were added

	CHECK, NO BORAX	1 POUND OF BORAX PER ACRE	2 POUNDS OF BORAX PER ACRE	5 POUNDS OF BORAX PER ACRE	10 POUNDS OF BORAX PER ACRE	20 POUNDS OF BORAX PER ACRE
Series 1. Borax-fertilizer mixtures in drills below the seed						
	gm.	gm.	gm.	gm.	gm.	gm.
Weights per pot 1......	17.68	0.00	21.46	0.00	0.00	0
Weights per pot 2......	20.50	11.72	8.37	0.00	0.00	0
Weights per pot 3......	9.74	19.06	22.16	0.00	15.02	0
Average..............	15.97	10.26	17.33	0.00	5.00	0
Series 2. Borax-fertilizer mixtures in drills above the seed						
Weights per pot 1......	10.93	0.00	16.06	17.32	4.28	0
Weights per pot 2......	13.13	9.00	8.74	0.00	0.00	0
Weights per pot 3......	14.45	14.07	20.67	10.74	2.28	0
Average................	12.84	7.69	15.16	9.35	2.19	0
Series 3. Borax-fertilizer mixtures broadcasted in first 3 inches of soil						
Weights per pot 1......	4.33	13.16	18.06	11.43	2.99	0
Weights per pot 2......	13.75	14.20	13.16	9.41	9.93	0
Weights per pot 3......	8.19	14.45	13.87	11.45	6.54	0
Average...............	8.76	13.93	15.03	10.86	6.49	0

Where the borax fertilizer mixtures were mixed with the upper 3 inches of soil (series 3) the roots of the 1 and 2-pound pots (pl. 8, fig. 2) appear like those of the check pot no. 116; those of the 5-pound pot no. 125 show the bushy development mentioned above, while considerable stunting and injury is evident in those of the 10-pound pot no. 127.

Dry weights of foliage

Table 6 gives the dry weights in grams of the foliage obtained from series 1, 2 and 3. In table 7 the average weight for each triplicate treatment is compared with that of the checks, which are given a value of 100. Too much

emphasis should not be placed upon these weights because of the large differences between triplicates, due to the uneven stand and growth of the corn. However, the differences caused by borax are very great and it is readily apparent from table 7 that the 10-pound applications caused a marked reduction in all cases, particularly in series 1 and 2; while the 20-pound application permitted no growth beyond the seedling stage.

Evidence of possible stimulation of corn plants due to borax

If the dry weights of series 3 alone are considered there is strong evidence of a marked stimulation of growth where borax was added at the rates of 1, 2 and 5 pounds per acre. A photograph of tops (pl. 8, fig. 1) taken at the time the plants were harvested on April 11, shows that the plants in pots 118 and 123 are larger than those in the check pot no. 116. Likewise the photograph of series 2 (pl. 7, fig. 1) exhibits an evident stimulation in the 1, 2 and 5-pound borax pots. But, according to the dry weights obtained, it is misleading in that respect (table 6) because the plants failed to grow in one of the pots with a 5-pound, as well as in one with a 1-pound borax application.

TABLE 7

Dry weights of corn foliage relative to the checks which are given a value of 100; based on the averages given in table 6

BORAX APPLIED PER ACRE	SERIES 1, IN DRILLS BELOW	SERIES 2, IN DRILLS ABOVE	SERIES 3, IN UPPER 3 INCHES
lbs.			
0	100	100	100
1	64	60	159
2	113	118	173
5	0	73	124
10	32	17	74
20	0	0	0

Although the growth in series 3 was much more uniform for a given treatment, the checks were smaller than those of the drilled series. Thus, when the three series are compared the evidence of a stimulating effect of borax seems to be rather doubtful. Since borax was always applied together with fertilizer the conditions of the experiment did not give much opportunity for borax stimulation to manifest itself. It is of interest to note that the broadcasted series of both the beans and the corn were alike in two respects. The dry weights of the foliage of the check plants were less than those of the checks of the drilled series, although the growth was more even than in the drilled series. Also, the dry weights from pots with low borax applications were considerably greater than those from the checks.

Results obtained with corn from adding lime, gypsum or manure to the borax-fertilizer mixtures

Hydrated lime, gypsum and well rotted manure were used with borax-fertilizer mixtures to determine whether or not they had a neutralizing or antagonizing action against borax injury to corn. Applications were at the rates of 1000, 1000 and 10,000 pounds per acre, respectively. The dry weights obtained are given in table 8. In series 4 the above materials were added with fertilizer and 5 pounds per acre of borax in drills below the seed. Thus the 5-pound borax pots of series 1 (table 6) constitute the checks for series 4. All of these check plants died. Therefore, if the death of the plants of the 5-pound borax pots of series 1 was caused entirely by the borax, as all observations indicated, then the growth obtained in series 4 must be attributed

TABLE 8

The dry weights of corn foliage obtained when either hydrated lime, gypsum or manure was added to borax-fertilizer mixtures

	HYDRATED LIME		GYPSUM		MANURE
	In drills	In first 3 inches	In drills	In first 3 inches	In first 3 inches
Series 4. Fertilizer and 5 pounds of borax per acre in drills below					
	gm.	gm.	gm.	gm.	gm.
Weight per pot 1..	7.86	11.31	3.08	9.82	4.66
Weight per pot 2..	8.65	0.00	13.08	0.00	11.41
Weight per pot 3..	9.72	7.44	9.70	10.55	13.37
Average..........	8.78	6.25	8.62	6.79	9.81
Series 5. Fertilizer and 10 pounds of borax per acre in drills below					
Weight per pot 1..	3.87	0.00			
Weight per pot 2..	13.37	0.00			
Weight per pot 3..	11.30	6.99			
Average..........	9.51	2.33			

to the antagonizing effect of the lime, gypsum and manure. In series 5, where the borax applications were at the rate of 10 pounds per acre, the effect of lime only was tested; and as all of the plants except one died in the pots used as checks in series 1, the growth obtained in series 5 must again be attributed to the antagonistic effect of the lime against borax injury.

Although characteristic stunting and banded bleaching were apparent in nearly all of the plants which grew in series 5, those of series 4 looked very nearly normal, especially in the manured pots.

Better growth was obtained in series 4, and particularly in series 5, where the lime was mixed directly with the borax-fertilizer mixtures, instead of with the upper 3 inches of soil. This was true also in similar treatments with beans, and indicates that the borax was converted into an insoluble and hence harmless calcium compound.

The results obtained with corn from using a commercial fertilizer containing borax[5]

The commercial fertilizer which was used contained somewhat less than 1 per cent of soluble boron compounds calculated as anhydrous borax and was applied in drills below the seed at the rates of 2000, 1000 and 500 pounds per acre. These applications are comparable to the 20, 10 and 5-pound borax applications in series 1 and the results obtained were much like those in series 1, as all of the plants which appeared soon died, with the exception of one plant in one of the pots to which the fertilizer was applied at the rate of 500 pounds per acre. This surviving plant was stunted and showed the characteristic banded bleaching.

Conclusions relative to the effect of borax upon corn

Corn plants appear to be particularly sensitive to borax when young but more resistant when older.

For this reason the toxic limit of borax for corn depends largely upon the method of application. The tests recorded above show that borax-fertilizer applications below the seed are much more liable to be toxic than those above or those which are mixed with the surface soil.

It was apparent that the application of 1 and 2 pounds per acre of borax was not harmful. The evidence indicates that 5 pounds is the largest amount of borax per acre that can be used on corn and that, even at this rate, injury may occur when the material is applied in drills below the seed.

The 10 and 20-pound treatments were toxic, under the conditions of these tests, and caused most of the seedlings either to fail to appear or to turn white and die. The few which grew were stunted and exhibited banded bleaching.

Lime and gypsum as well as manure appeared to neutralize some of the toxic effects of borax.

The results obtained from using a ready-mixed commercial fertilizer containing borax were much the same as those resulting from the use of borax-fertilizer mixtures made from tested chemicals.

EFFECT OF BORAX UPON THE GROWTH OF BEANS

The amount and methods of application of fertilizer and borax to beans were the same as are described for corn on page 83.

The seedling stage

Ten days after the beans had been planted (February 5) many of the plants in the 0 (checks), 1 and 2-pound-per-acre borax lots were about 3 inches high,

[5] The same fertilizer as was used for potatoes (p. 83).

those in the 5-pound borax lots were smaller, and none had appeared in the pots containing borax at the rates of 10 and 20 pounds per acre. Later a few seedlings appeared in the highest two borax applications but they were of a yellowish, sickly appearance. If the affected cotyledons were finally drawn out of the soil they often remained incrusted with the seed-coat and did not open. This effect upon germination is best shown in table 9, which gives the total number of seedlings appearing previous to March 1. Since 5 seeds were planted in each pot a total of 15 per treatment indicates perfect germination. The effect of increasing amounts of borax is apparent in series 3 in which the borax-fertilizer mixtures were broadcasted and mixed with the upper 3 inches of soil. It is especially striking in the drilled series no. 1 and 2 in which no growth appeared in the 20 and very little in the 10-pound lots of borax.

Since all seedlings were removed that appeared after the first three, an opportunity was given to examine the roots of those showing borax injury in their aerial parts. Invariably the tap-roots were found to be destroyed and often also the tips of some of the larger laterals.

TABLE 9

Total number of bean seedlings which appeared where borax-fertilizers mixtures were applied in drills below (series 1); in drills above (series 2); and mixed with the first 3 inches of soil (series 3)

POUNDS OF BORAX PER ACRE	SERIES 1						SERIES 2						SERIES 3						NO ADDITION TO SOIL
	0	1	2	5	10	20	0	1	2	5	10	20	0	1	2	5	10	20	
Seedlings per individual pot 1	4	5	5	2	3	0	5	3	3	4	1	0	5	3	5	4	5	4	4
2	3	4	3	4	0	0	4	4	3	3	1	0	5	5	5	5	5	3	5
3	4	4	3	5	4	0	5	4	5	4	1	0	5	5	5	5	5	4	4
Total per treatment	11	13	11	11	7	0	14	11	11	11	3	0	15	13	15	14	15	11	13

Effect of borax upon foliage growth of beans

Although some of the plants in the pots of the highest borax applications in series 3 remained alive until harvested (April 22) they were very much stunted and of a whitish to yellowish appearance. Many of the older leaves fell off. Figure 2 of plate 9 shows these plants in the foreground with the plants of the 10, 5 and 2-pound-per-acre borax lots in the background. A decreasingly abnormal appearance may be clearly seen with decreasing amounts of borax.

The injuries due to borax appeared first on the margins of the first two leaves, particularly the tips. In the larger borax applications the entire leaf soon turned yellow, then white, followed by a killing of the tissues which progressed from the margins inward. New leaves either failed to appear or were very much stunted and almost devoid of chlorophyl. The two lower pots of figure 1, plate 9, illustrate these extreme cases. The two upper pots

show plants in which there is somewhat less injury as evidenced by larger, new leaves containing a small amount of chlorophyl. These correspond to the second row in figure 2. The next row in figure 2 shows still larger new leaves which are bleached only at the tips. The rear row received borax at the rate of 2 pounds per acre and has new leaves which are normal and old leaves which were very slightly killed at their margins. The checks and 1-pound borax lots showed none of the injury described above. Series 1 and 2 exhibited a sharper gradation of borax injury than series 3. Thus, although the checks and the 1 and 2-pound borax lots appeared much the same in all cases, the plants of the 5-pound lot of the drilled series, no. 1 and 2, showed nearly as much injury as those of the 10-pound lot of the broadcasted series no. 3. In a similar way plants of the 10-pound drilled lots were much like those of the 20-pound broadcasted applications. No growth appeared in pots which received 20 pounds of borax per acre in drills. In general the plants of the 5-pound borax lots recovered somewhat but always exhibited considerable injury, particularly on series 1. A photograph taken February 23 shows the 10-pound borax pots of the three series (pl. 10, fig. 1) and demonstrates that borax added in drills below the seed was the most toxic, followed by that added in drills above, while the least ill effects were obtained when it was mixed with the upper 3 inches of soil.

The sprouting roots come into contact with the borax-fertilizer mixture very early when it is applied in drills below, and this checks the growth or kills the plants at an early stage. The observations show that a young plant is especially sensitive to borax. It is also probable that after diffusion of the material the toxic action of the borax was partly inhibited on account of its dilution or by some property of the soil. Thus, by April 2 all of the plants in the 10-pound borax lots of series 1 were dying, except one which had become larger than the others before showing injury. At the time of harvesting it was growing rapidly, had become dark green and had set large pods. Its roots had probably come into contact with considerable borax, as all of the other plants of this pot died when young, and the borax during a period of 2 months had surely become diffused throughout the soil contained within the solid, glazed pot. An equally striking example occurred in one of the pots in which corn had been planted.

By the time that the beans were harvested, the plants which were not too severely injured had produced large, well filled pods. In some cases the chlorophyl of the pods was bleached out, as is easily seen in pot 31 (pl. 11, fig. 3) which received a 10-pound-per-acre borax application in drills below the seed. Pot 51 received the same amount of borax mixed with the upper 3 inches of soil and its plants show less injury, while the plants of pot 10, which received a 5-pound-per-acre borax application, show still less injury.

Representative plants, including their root systems, are shown in plates 12 and 13. In plate 12 the borax applications from right to left are at the rates of 0, 1, 2, 5 and 10 pounds per acre in drills above the seed. In plate

13 the order of the broadcasted borax applications is the same; the photographs show the insignificant growth in the pot containing borax at the rate of 20 pounds per acre.

Effect of borax upon the roots of beans

The plants had begun to ripen slightly when harvested and the root systems showed a marked decrease with increasing amounts of borax above the 2-pound applications. This may be noted in plates 12 and 13, as well as a tendency toward a bushy development of finer roots grown in the presence of 1 and 2 pounds of borax per acre. The decrease in root systems with increasing amounts of borax was accompanied by an increase, relatively, in the number of laterals which had been browned and killed. Cases of slight injury were revealed by the browned and broken tap-roots. The tap-roots also were the most injured portions in the poisoned seedlings which were removed.

Moreover, the root nodules, which were well developed in the checks and 1 and 2-pound borax lots, were markedly reduced in size and number with increasing amounts of borax (pl. 12 and 13).

Dry weights of foliage of beans

The dry weights of the aerial portions of the bean crop are shown in tables 10 and 11. It may be noted that the triplicate determinations check satisfactorily. A comparison of the average dry weights is made in table 12, in which the weights are referred to the checks which are given a value of 100. These figures point out more definitely the injurious effects of borax which were noted during the growing season. Thus the values for the 1 and 2-pound-per-acre borax lots are much alike in the three series. However, there is a marked reduction with the 5-pound borax applications, which averages 46 per cent in the drilled series and 16 per cent in the broadcasted series no. 3. The crops with higher borax applications were practically complete failures.

About 3 weeks before the harvesting there was some evidence of stimulation in the plants of the 1 and 2-pound borax lots. At the time of harvesting appearances of stimulation had disappeared and none, on the average, is shown by the dry weights.

Influence of water content of soil upon the growth of beans in borax-treated pots

The above tests were all conducted with soil at an optimum water content of 19.2 per cent. In order to ascertain somewhat the influence of soil-moisture conditions upon borax injury, two additional series were planted on March 17. The soil of series 8 was held at a moisture content of 15.2 and that of series 7 at 30.4 per cent, equivalent to 40 and to 80 per cent, respectively, of the maximum water-capacity of the soil.

TABLE 10

The dry weights of bean foliage from pots to which fertilizer and definite amounts of borax were added

		Check, no borax	1 pound of borax per acre	2 pounds of borax per acre	5 pounds of borax per acre	10 pounds of borax per acre	20 pounds of borax per acre
Series 1. Borax-fertilizer mixtures in drills below the seed							
		gm.	gm.	gm.	gm.	gm.	gm.
Weight per pot	1.......	25.39	22.86	20.75	*	15.78	0
	2.......	26.83	26.30	24.43	17.81	0.50	0
	3.......	*	24.15	22.93	15.88	5.52	0
Average...............		26.21	24.44	22.70	16.85	7.20	0
Series 2. Borax-fertilizer mixtures in drills above the seed							
Weight per pot	1.......	23.62	22.36	23.80	7.03	*	0
	2.......	22.48	23.86	26.10	9.07	0.09	0
	3.......	25.84	22.90	21.71	14.76	0.80	0
Average...............		23.98	23.04	23.87	10.29	0.50	0
Series 3. Borax-fertilizer mixtures in upper 3 inches of soil							
Weight per pot	1.......	Diseased	25.07	25.11	16.67	8.80	0.69
	2.......	18.07	23.43	21.25	13.97	9.24	0.25
	3.......	18.83	20.70	21.53	15.78	10.82	0.40
Average...............		18.50	23.07	22.63	15.47	9.62	0.45

The triplicate control pots (no addition to the soil) gave dry weights of 18.87, 16.82 and 15.45 gm., respectively.

* Used for chemical tests.

TABLE 11

The dry weight of bean foliage obtained when either hydrated lime, gypsum or manure was added to borax-fertilizer mixtures

		Hydrated lime		Gypsum		Manure
		In drills	In upper 3 inches	In drills	In upper 3 inches	In upper 3 inches
Series 4. Fertilizer and 5 pounds of borax per acre in drills below						
		gm.	gm.	gm.	gm.	gm.
Weight per pot	1...........	18.10	18.17	15.89	18.38	0.95
	2...........	20.13	22.00	11.11	17.55	2.35
	3...........	18.56	8.10	*	17.95	*
Average.....................		18.93	16.09	13.50	17.96	1.10
Series 5. Fertilizer and 10 pounds of borax per acre in drills below						
Weight per pot	1...........	12.48	12.02			
	2...........	15.76	16.26			
	3...........	15.59	16.56			
Average.....................		14.61	14.94			

As the previous work indicated that the toxic limit for borax was between 2 and 5 pounds per acre, the borax applications for series 7 and 8 were made at the rates of 0, 2, 4, 6 and 8 pounds per acre. Table 13 shows that increasing amounts of borax considerably reduced the total number of seedlings which appeared in series 8. In series 7 in which the water content of the soil was twice as great, the borax applied had no marked effect on germination. The photographs taken 28 days after planting (pl. 11, fig. 1 and 2) show that the characteristic borax injury is also more marked in the series with the drier soil.

TABLE 12

Weights of bean foliage relative to the checks, which are given a value of 100; based on the averages in tables 10 and 11

APPLICATION OF BORAX PER ACRE	NONE IN CHECK	1 POUND	2 POUNDS	5 POUNDS	10 POUNDS	20 POUNDS
Borax-fertilizer mixtures in drills below (series 1)	100	93	87	64	27	0
Borax-fertilizer mixtures in drills above (series 2)	100	97	99	43	2	0
Borax-fertilizer mixtures in top 3 inches (series 3)	100	125	122	84	52	2

TABLE 13

Total number of bean seedlings which appeared in pots of series 7 and 8

POUNDS OF BORAX PER ACRE	SERIES 7, 30 PER CENT OF MOISTURE						SERIES 8, 15 PER CENT OF MOISTURE					
	0	2	4	6	8	4½*	0	2	4	6	8	4½*
Seedlings per individual pot 1	4	4	5	4	5	5	5	3	3	4	3	5
Seedlings per individual pot 2	5	5	4	5	2	5	5	3	3	2	1	5
Seedlings per individual pot 3	3	5	†	3	5	5	4	4	4	5	1	5
Total per treatment	12	14	12	12	12	15	14	10	10	11	5	15

* A commercial fertilizer used which contained about 1 per cent of borax.

† Lost.

Dry-weight determinations were not made in the case of series 7 and 8, as some of the leaves were used for chemical determinations by Mr. Jones. It was found, quantitatively, that increasing applications of borax not only caused greater leaf injury but also caused a more concentrated accumulation of boron in the leaves, while those of the checks did not contain boron.

Results obtained with beans from the application of a commercial fertilizer which contained borax

In order to ascertain whether the injury obtained with a fertilizer which contained borax was similar to that obtained with a fertilizer to which borax was added, a set of pots were treated with a 4–8–6 commercial fertilizer which

contained about 1 per cent of borax.[6] In the first set, in which the soil-moisture content was 19.2 per cent, pots were treated in duplicate with the commercial fertilizer at the rates of 2000, 1000 and 500 pounds per acre in drills below. These, then, were comparable to the 20, 10 and 5-pound borax applications in series 1. The same type of injury developed in the plants in both cases but it was more pronounced in those of the pots receiving the commercial fertilizer and the corresponding dry weights were less. This fertilizer was applied at the rate of 450 pounds per acre in series 7, of which the soil moisture content was 30.4 per cent, and in series 8, the soil of which contained 15.2 per cent of water. As may be seen in the photographs (pl. 11, fig. 1 and 2) the commercial fertilizer (last row at right) caused more injury than the 4-pound borax lots (third row from left) and nearly as much as the 6-pound lots (fourth row from left). Leaves from the plants where the commercial fertilizer was added contained more boron than those of the 4-pound borax pots. Thus it was evident that borax-fertilizer mixtures were less toxic than a commercial fertilizer containing an equivalent amount of borax. Since more borax accumulated in the leaves in the latter case it seems that this super-toxicity was due not to some deleterious substance other than borax but possibly to a slower dissolving and leaching away of the naturally incorporated borax.

The use of lime, gypsum and manure with borax-fertilizer mixtures for beans

In the bean studies, series 4 received a fertilizer mixture, carrying at the rate of application, 5 pounds of borax per acre in drills below the seed together with an addition of hydrated lime or of gypsum at the rate of 1000 pounds per acre. In one set of pots the calcium salt was mixed with the fertilizer and in the other it was mixed with the upper 3 inches of soil.

As early as February 23 the recorded notes state that lime, particularly when mixed with the fertilizer, was showing a beneficial effect which is readily apparent in a photograph taken March 3 (pl. 10, fig. 2). At the time of harvesting, however, the limed pots of the 5-pound borax lots looked much the same as those which were not limed and the dry weights (table 11) are much alike in both cases.

The early effect of lime added to 10-pound borax lots (series 5) was more marked than in series 4 (pl. 10, fig. 1) where borax was present at the rate of 10 pounds per acre. The limed plants of series 5 continued to show a superiority, in both foliage and roots. Their dry weights (table 11) are more than 100 per cent greater than the comparable unlimed plants of series 1. However, in no case did the lime neutralize more than a portion of the toxicity caused by borax. The neutralizing effect which occurred may have been due to an antagonism between the lime and the borax, but was more probably due to the conversion of the borax into insoluble and hence harmless compounds.

[6] The same as was used on potatoes (p. 83).

Gypsum appeared at first (pl. 10, fig. 2) to render some benefit, but the effect disappeared and is not indicated by the dry weights obtained (table 11).

In the manured pots a number of the plants were attacked by a parasite. The others suffered excessively from borax injury and the results indicated that the manure increased the toxicity of borax.

Conclusions from the tests with beans

Borax applied at the rate of 2 pounds per acre caused no injury to the growth of beans while with a 4-pound application the characteristic injury and stunting of growth was quite marked. Under the conditions of the experiment, therefore, the toxic limit for this plant was in the neighborhood of 3 pounds of borax per acre when applied in drills. When broadcasted the toxic limit was about 5 pounds per acre. Most of the plants in the 10-pound and all of those of the 20-pound-per-acre borax applications either died or failed to appear above the soil.

Young bean plants are especially sensitive toward borax injury. For that reason borax-fertilizer applications in drills, particularly in drills below the seed, are more toxic than broadcasted applications, since the young roots come into contact with dissolved borax earlier and since the borax solution is more concentrated at first, when applied in drills.

Given amounts of borax which were toxic toward germination and growth in soil held at a 30 per cent moisture content were more toxic in soil containing half as much water.

In a comparison of borax-fertilizer mixtures with a commercial fertilizer, containing an equivalent amount of borax, the same type of injury occurred in both cases and the commercial fertilizer was more toxic. Since the leaves in the latter case contained a greater quantity of boron it is believed that the super-toxicity of the commercial fertilizer was not due to some additional deleterious substance, but rather to a slower dissolving and leaching away of the naturally incorporated borax which would cause the young roots to come into contact with borax for a greater length of time.

Hydrated lime neutralized a part of the toxic effect of borax, particularly when mixed and applied in drills with the borax-fertilizer mixtures. The applications of gypsum and manure did not retard the toxicity of borax to any appreciable extent.

GENERAL SUMMARY

Plants were uninjured where fertilizer mixtures made from borax-free chemicals were applied to soil in pots in which potatoes, corn and beans were grown. These crops were injured where the pots contained the same soil and the same fertilizer mixtures in like quantity, provided sufficient amounts of borax were added with the fertilizer. The same types of injury were produced, in somewhat greater degree, when a commercial fertilizer carrying equivalent amounts of borax was applied.

Corn and beans were more suceptible to the injurious effects of borax than were potatoes. Under the conditions of the experiment, anhydrous borax at the rate of 3 pounds per acre was the largest amount that could be applied in drills with safety to beans. The limit for corn is somewhat under 5 pounds, and for potatoes slightly above 5 pounds per acre. Borax applied with the fertilizer below the seed or seed-piece proved more toxic in all cases than where applied above in like manner. Mixing the borax and fertilizer with the soil decreased the injury and slightly raised the amount that could be applied per acre with safety.

Evidence was obtained that applications of lime prevented some of the injury to potatoes. The tests with gypsum and manure were not conclusive with this crop. All three of these materials seemed to reduce the toxic effects on corn. Lime was beneficial with beans, but gypsum and manure did not show any appreciable influence.

The above results were all obtained with soil at an optimum water content of 19.2 per cent. A subsequent test with beans showed that more injury occurred where the soil moisture was maintained at 15.2 per cent than where it was 30.4 per cent.

The only indication of possible stimulation due to the presence of small amounts of boron occurred with corn, but the evidence was inconclusive.

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PLATE 1

FIG. 1. Potato plants photographed March 29. Pots 56 and 57 received borax at the rate of 10 pounds per acre in drills above the seed-pieces. The plants were stunted early in pot 56. They grew normally at first in pot 57 but are beginning to develop excessive leaf injury. The plants of pot 73 have leaves which are normal.

FIG. 2. Potato leaves photographed March 29. A normal potato leaf at the left followed by increasing amounts of injury in the next two leaves and in the last two at the right. The third and fourth leaves from the right are from the upper part of an affected plant.

FIG. 3. Potato plants photographed April 22. Plants in pot 13 (20 pounds of borax per acre in drills below) were stunted while young. Those in pot 25 (10 pounds of borax per acre in drills above) grew normally at first and then developed considerable leaf injury. Leaf injury is excessive in the case of pot 60 (20 pounds of borax per acre mixed with the upper 3 inches of soil).

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PLATE 2

FIG. 1. Potato plants photographed March 9. Triplicately treated pots of series 1 (borax-fertilizer mixtures in drills below the seed-pieces). Borax applications at the rates of 0, 2, 5, 10 and 20 pounds per acre from right to left.

FIG. 2. Potato plants photographed March 9. Plants of series 2 (borax-fertilizer mixtures in drills above the seed pieces) arranged as in figure 1 above.


SERIES 1
SERIES 2

PLATE 3

FIG. 1. Potato plants photographed March 9. Triplicately treated pots of series 3 (borax fertilizer mixtures broadcasted in the second 3 inches of soil). Borax applications at the rates of 0, 2, 5, 10 and 20 pounds per acre from right to left.

FIG. 2. Potato plants photographed March 9. Plants of series 4 (borax-fertilizer mixtures in upper 3 inches of soil) arranged as in figure 1 above.


SERIES 3


SERIES 4
2

PLATE 4

FIG. 1. Potato plants photographed March 29. Representative pots from series 2 (borax-fertilizer mixtures in drills above the seed-pieces). Borax applications at the rate of 0, 2, 5, 10 and 20 pounds per acre from left to right.

FIG. 2. Representative plants of series 1 (borax-fertilizer mixtures in drills below the seed-pieces) arranged as in figure 1 above. Photographed on March 29.

FIG. 3. Representative plants of series 3 (borax-fertilizer mixtures broadcasted in second 3 inches of soil) photographed March 29 and arranged as in figure 1 above.

EFFECT OF BORAX ON POTATOES, CORN AND BEANS

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PLATE 4

PLATE 5

FIG. 1. Potato plants photographed March 29. Representative pots from series 4 (borax-fertilizer mixtures broadcasted in upper 3 inches of soil). Borax applications at the rates of 0, 2, 5, 10 and 20 pounds per acre from left to right.

FIG. 2. Potato plants photographed March 29. Borax at the rate of 10 pounds per acre in drills below with hydrated lime (pot 88), with finely ground limestone (pot 84) and with fertilizer only (pot 10). Borax at the rate of 5 pounds per acre in drills below, with fertilizer only (pot 9), with finely ground limestone (pot 62) and with gypsum (pot 69).

FIG. 3. Potato roots and tubers from representative pots of series 2 (pl. 4, fig. 1), photographed April 22, arranged as in figure 1 above.

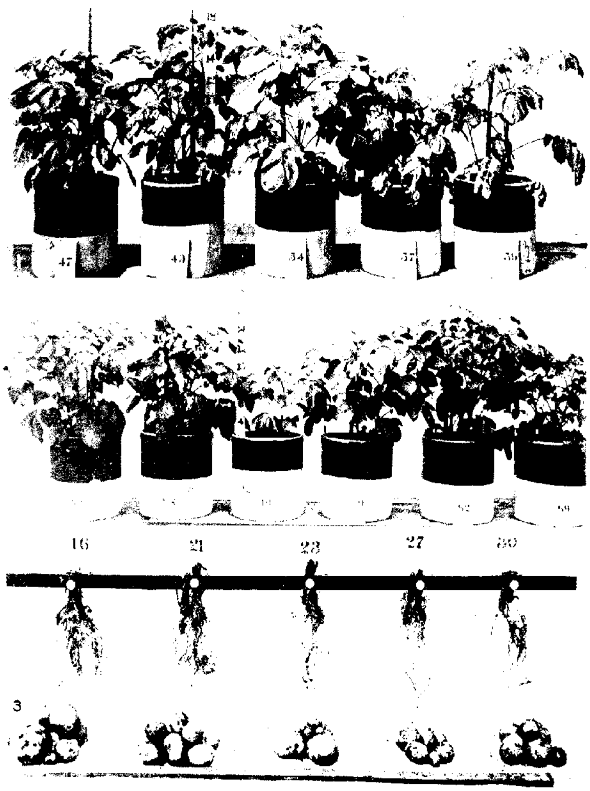


PLATE 6

FIG. 1. Potato roots and tubers photographed April 22. Representative pots from series 1 (pl. 4, fig. 2). Borax applications were at the rates of 0, 2, 5, 10 and 20 pounds per acre from left to right.

FIG. 2. Potato roots and tubers from representative pots of series 4 (pl. 5, fig. 1) photographed April 22 and arranged as in figure 1 above.

FIG. 3. Potato roots and tubers from representative pots of series 3 (pl. 4, fig. 3) photographed April 22 and arranged as in figure 1 above.

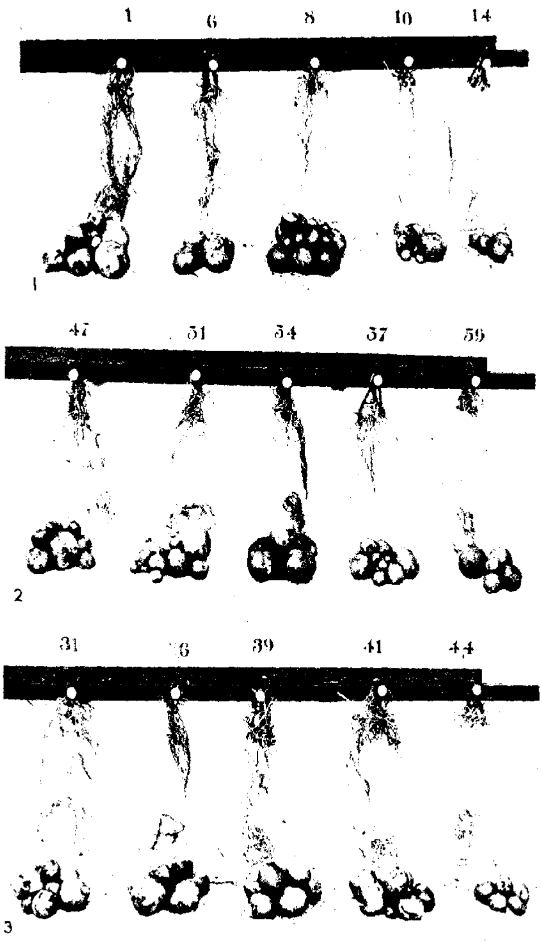
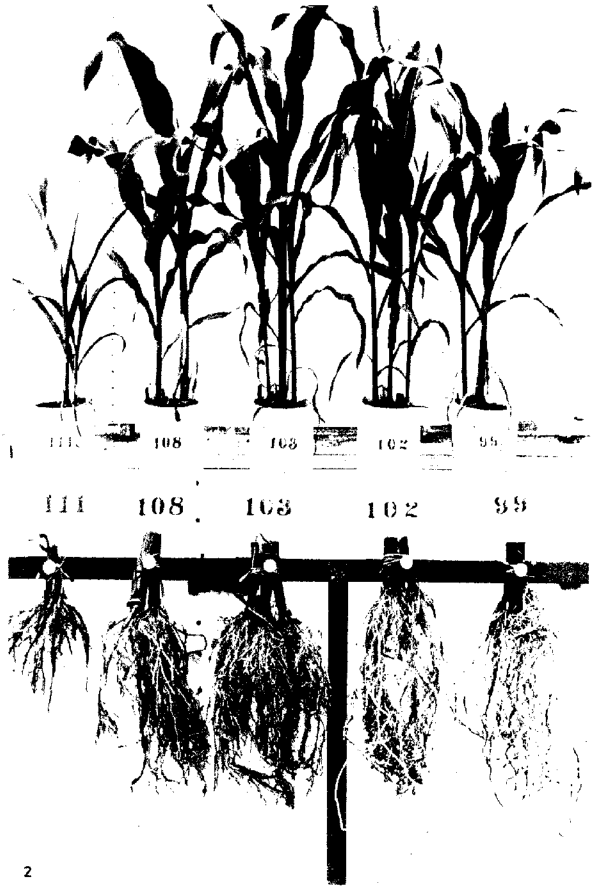

1 6 8 10 14
1
47 51 54 57 59
2
31 39 41 44
3

PLATE 7

FIG. 1. Corn plants photographed March 11. Representative pots of series 2 (borax-fertilizer mixtures in drills above the seed). Borax applications at the rates of 0, 1, 2, 5 and 10 pounds per acre from right to left.

FIG. 2. Roots of plants shown in figure 1 above, photographed March 11.



2

PLATE 8

FIG. 1. Corn plants photographed March 11. Representative pots of series 3 (borax-fertilizer mixtures in upper 3 inches of soil). Borax applications at the rates of 0, 1, 2, 5 and 10 pounds per acre from right to left.

FIG. 2. Roots of plants shown in figure 1 above, photographed March 11.

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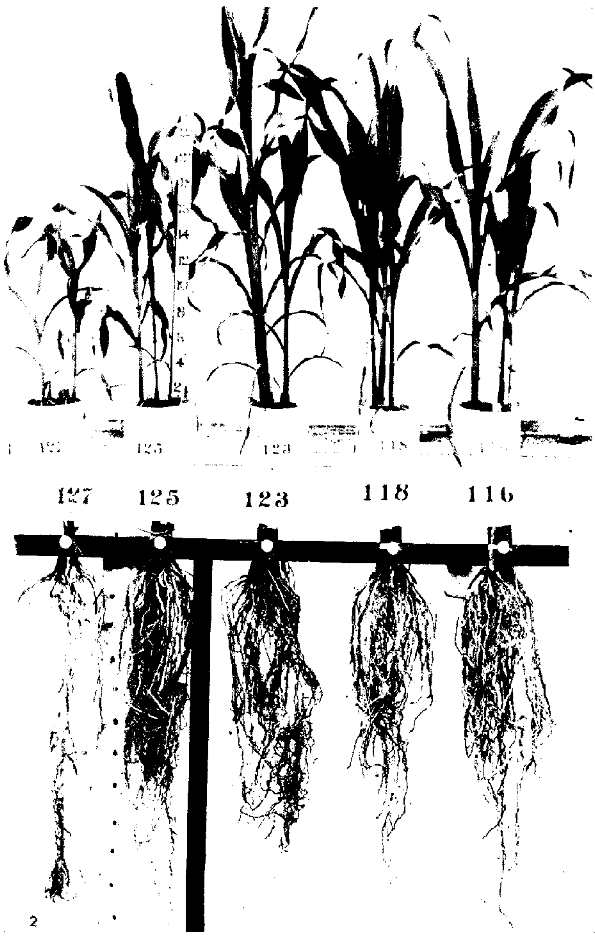

PLATE 9

FIG. 1. Bean plants photographed February 23. Borax at the rate of 10 pounds per acre was mixed with the upper 3 inches of soil in upper right-hand pot; in drills above the seed in upper left-hand pot; and in drills below the seed in lower left-hand pot. A commercial fertilizer containing about 1 per cent of borax was added at the rate of 500 pounds per acre to the lower right-hand pot.

FIG. 2. Bean plants photographed February 23. Borax, mixed with the upper 3 inches of soil, was applied at the rates of 20 pounds per acre in the first row at the bottom, 10 pounds per acre in the second, 5 pounds in the third and 2 pounds in the fourth row.

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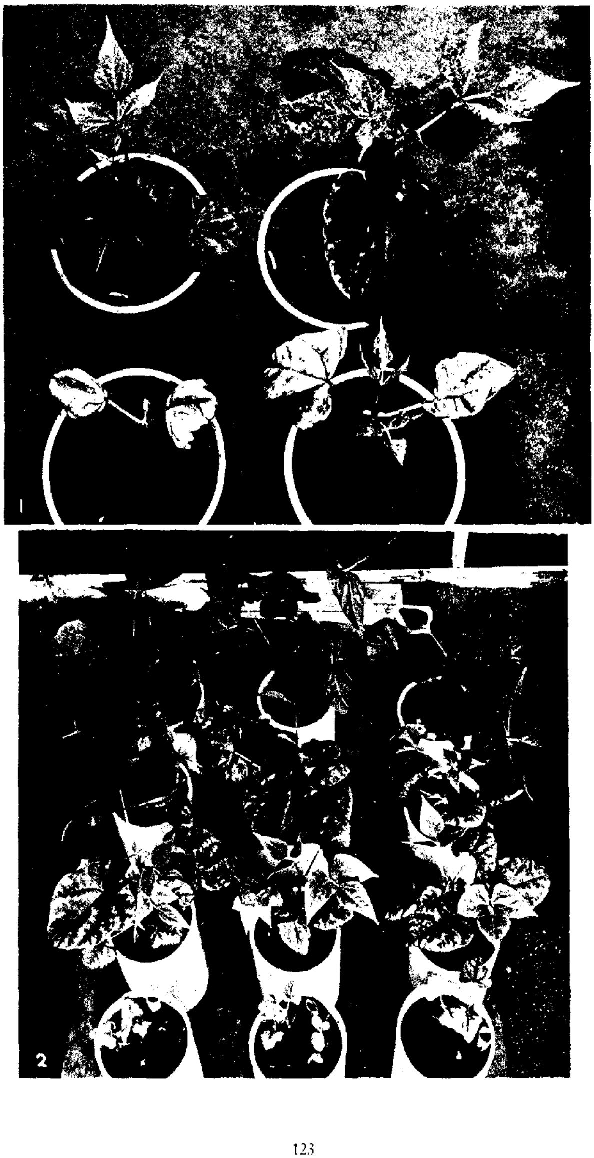


PLATE 10

FIG. 1. Bean plants photographed February 23. Borax applied at the rate of 10 pounds per acre as follows, beginning with the row (triplicate treatments) at the left; in the upper 3 inches of soil, in drills above, in drills below, in drills below with hydrated lime, and in drills below with the lime in the upper 3 inches.

FIG. 2. Bean plants photographed February 23. Borax applied at the rate of 5 pounds per acre in drills below together with additions as follows, beginning with the row at the left; gypsum mixed with the upper 3 inches, gypsum mixed with the borax-fertilizer mixture, borax-fertilizer only, hydrated lime mixed with the borax-fertilizer mixture, and hydrated lime mixed in the upper 3 inches.


10
LIME
1
GYPSUM
5
LIME
2

PLATE 11

FIG. 1. Bean plants photographed April 22. Series 7 received fertilizer in drills below together with borax at the rates of (left to right) 0, 2, 4, 6 and 8 pounds per acre. The pots in the row at the right received $4\frac{1}{2}$ pounds of borax per acre in a commercial fertilizer containing about 1 per cent of borax.

FIG. 2. Series 8 is the same as series 7 except that the soil was maintained at a moisture content of 15 per cent in series 8 and 30 per cent in series 7.

FIG. 3. Pot 31 received 10 pounds of borax per acre in drills above the seed, pot 51 the same amount mixed with the upper 3 inches of soil, and pot 10, 5 pounds per acre in drills below the seed. The pods as well as the leaves were bleached in pot 31.


SERIES 7
SERIES 8
10

PLATE 12

FIG. 1. Bean plants photographed April 12. Representative pots of the triplicate treatments of series 2 in which the fertilizer was in drills above the seed, together with the borax per acre as follows: none (pot 19), 1 pound (pot 22), 2 pounds (pot 27), 5 pounds (pot 28), 10 pounds (pot 33).

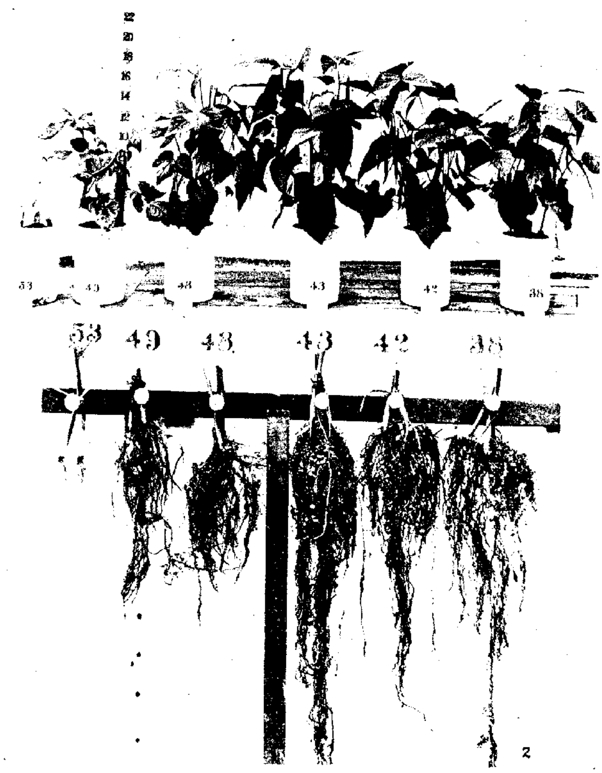
FIG. 2. Roots of the plants shown in figure 1, photographed April 12.


2

PLATE 13

FIG. 1. Bean plants photographed April 12. Representative pots of the triplicate treatments of series 3 in which the fertilizer was mixed with the upper 3 inches of soil together with borax at the following rates per acre: none (pot 38), 1 pound (pot 42), 2 pounds (pot 43), 5 pounds (pot 48), 10 pounds (pot 49), and 20 pounds (pot 53).

FIG. 2. Roots of the plants shown in figure 1, photographed April 12.


53
49
48
43
42
38
2

SOIL ACIDITY AND BACTERIAL ACTIVITY[1]

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INTRODUCTION

Just how and why soils become acid is a problem that has not yet been definitely solved. Neither is the effect of reaction upon the activity of soil organisms clearly understood. But is has been fairly well established that the process of nitrification once thought to be absent in acid soils, does proceed to an appreciable extent. In fact nitrification is perhaps sufficient for normal crop production, in most cases, provided the supply of organic matter is adequate. The process of ammonification which of course must precede nitrification is carried on by so many classes of organisms that it is not usually a limiting factor in crop production in either acid or sweet soils, under aerobic or anaerobic conditions.

In practically all soils there must be two analytical processes, the decomposition of organic matter, and the disintegration of minerals. The importance of microorganisms in bringing about these processes is too obvious to need comment. While these processes are occurring, plant growth also takes place. The general tendency of plant growth has been found to be to keep the nutrient solution nearly neutral. Crop production therefore doubtless has a tendency to prevent soils from becoming acid in reaction, while the leaching of bases has the opposite effect.

The cultivation of soils for crop production of course encourages leaching, stimulates bacterial activity, and on the whole in this indirect way must tend to produce acid soils.

In mineral disintegration, with the accompanying interchange of ions, both acids and bases must be set free. Similar effects are produced when organic matter is broken down. But changes in the organic portion of the soils must occur under favorable conditions much more rapidly than changes in the mineral portion. The acids and carbon dioxide produced in organic decay hasten mineral disintegration, and therefore increase the availability of mineral

[1] Part of the results of this study on "Soil Acidity and Bacterial Activity" has already been published. Two papers, "The Effect of Organic Matter on Soil Reactions. I," and "The Activity of Soil Acids" were published in Soil Science (6, 7), another paper "Nitrification in Acid Soils" is in press at the Iowa Agricultural Experiment Station. This paper is the first part of a thesis presented to the graduate faculty of the Iowa State College of Agriculture in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

plant-food. But though minerals are put into solution by these processes, there is also a compensating effect, in that organic decomposition products are capable of forming insoluble compounds with the minerals disintegrated and thus may prevent or at least retard the loss of the minerals by leaching.

One fact to be kept in mind in connection with both organic acids and with bases, is that so far as available data indicate, these compounds do not remain long as such. Oxidation changes convert the nitrogen bases to nitric acid and the organic acids to carbon dioxide. Only the nitric acid produced, therefore, is capable of causing a permanent direct effect upon soil reaction. Mineral bases and acids, on the other hand, are permanently removed from the soil primarily by leaching. The portion used by the plant may be expected to be returned to the soil, at least in part.

It may be observed, too, that practical experience demonstrates that soils containing sufficient organic matter remain more productive for a longer time than those soils which are low in organic matter. Loss of organic matter is likely to result in a sour, soggy, infertile soil, which does not respond to tillage or commercial fertilizer. Muck and peat soils are notable exceptions but largely because mineral elements, such as potassium and other bases, were never present. And again, such soils occur only under those conditions which favor a large production of organic acids, and prevent complete oxidation. These soils, therefore, are often highly acid, and this condition is undoubtedly due mainly to organic acids. But by way of contrast it must be observed that sandy soils and heavy clays, which do not contain sufficient organic matter to produce an appreciable acidity, are often highly acid and nonproductive.

In this work one heavy silt loam soil, one soil somewhat sandy, both low in organic matter, and a loam soil rather high in organic matter were used, for the purpose of studying the changes which occur, the rate of change, and to some extent the final products of the reactions.

HISTORICAL

Previous investigations of the effect of organic matter upon the reaction of soils is very limited in amount and application. White (8), Skinner and Beatty (3), Miller (2) and Stephenson (6) found no positive evidence that the decay of organic matter in ordinary soils under conditions which would be favorable to crop production, produced any appreciable increase in the lime requirement of the soil. No argument is necessary, of course, to establish the fact that the large production of nitric acid would increase the acid reaction of the soil or use up bases rapidly if they were present.

THE PLAN OF THE EXPERIMENT

In a previous publication (6) the effect of the decomposition of albumin, casein, starch, blood, dextrose, alfalfa, and ammonium sulfate on the reaction of two soils was studied. Further work along this same line is reported here,

with organic materials of more general use such as farm manure, cottonseed meal, horse manure, timothy hay, clover hay, green timothy and green clover. Opportunity is thus afforded for comparing the green and the more matured dried materials.

Two of the same soils used in the earlier work were employed, one rather sandy and light in color, the other dark and fairly rich in organic matter, and of the loam type. Applications of the various materials were made at the rate of 10 tons per acre of air-dried material, on the basis of 2,000,000 pounds of soil per acre. The coarse materials were ground and thoroughly mixed with the soils, in 1-gallon earthenware jars. Samplings were made at intervals of 2, 5, 10, 15, and 22 weeks, respectively. Two series were run, one limed and the other unlimed. Determinations were made at each sampling for the ammonia, nitrates, acidity, and residual carbonates, since these are directly connected with the effect of materials on the soil reaction. A test was made at the second sampling, for the soluble non-protein nitrogen present in one of the soil types. This test should throw some light on the question of the possibility of any accumulation of soluble products of protein decomposition, other than nitrates and ammonia, and should also show whether there is any correlation between these products and the quantity of nitrates or ammonia present in soils.

AMMONIFICATION

The quantity of ammonia was determined by the aeration method, potassium carbonate being used to liberate the ammonia. Incidentally it may be said that experience at the Iowa Agricultural Experiment Station with this method would lead to the conclusion that those workers who have found the method unsatisfactory, must have experienced a faulty manipulation. The secret of successful operation of the method, is that the aeration must stir the soil completely to the bottom of the containing flask. The results of the ammonia determinations are given in table 1.

It may be observed that there is very little accumulation of ammonia with any of the treatments except the cottonseed meal. It has shown the greatest accumulation of ammonia at the first sampling and a greater accumulation when the soil was untreated, than when it was limed, both of which results agree with work done previously (6) with highly nitrogenous materials. There is too small an accumulation of ammonia on the untreated soils to show marked differences between the limed and the unlimed soils. The same may be said of most of the other treatments, though there is a greater amount of ammonia in the unlimed soils where green manures were added. The greatest amount of ammonia is found in nearly all cases at the first sampling before nitrification is well started. There is quite a marked difference in the two soils, noticeable where the cottonseed meal is used, in that the amount of ammonia throughout the test remains high on the unlimed sandy soil, while on the humus soil nitrification seems to have just about kept pace with

ammonification even in the absence of lime. This result lends support to the belief that soils containing sufficient organic matter are more active bacteriologically, and likewise usually more productive, than soils containing less organic matter even when the total time requirement is much greater for the organic soils.

The amount of ammonia produced may depend upon several factors. But when conditions are favorable for nitrification the ammonia is changed to nitrates almost as rapidly as produced.

TABLE 1

Amount of ammonia at the end of each period

TREATMENT	FIRST SAMPLE, 2 WEEKS		SECOND SAMPLE, 5 WEEKS		THIRD SAMPLE, 10 WEEKS		FOURTH SAMPLE, 15 WEEKS		FIFTH SAMPLE, 22 WEEKS		AVERAGES	
	No lime	Lime	No lime	Lime	No lime	Lime	No lime	Lime	No lime	Lime	No lime	Lime
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Humus soil:												
Soil alone	11.8	14.0	11.2	8.4	16.8	11.2	11.2	14.0	11.2	8.4	13.4	11.1
Cottonseed meal	302.4	285.6	268.8	61.6	98.0	22.4	86.8	19.6	32.0	14.0	157.6	80.6
Manure	8.4	5.6	11.2	8.4	11.2	11.2	14.0	11.2	14.0	11.2	11.7	9.6
Timothy hay	5.6	8.4	11.2	11.2	16.8	11.2	11.2	5.6	11.2	11.2	11.2	9.5
Clover hay	19.6	11.2	8.4	5.6	11.2	11.2	5.6	11.2	11.2	11.2	11.2	10.1
Green timothy	44.8	11.2	14.0	5.6	16.8	11.2	11.2	11.2	11.2	11.2	19.6	10.1
Green clover	33.6	14.0	16.8	5.6	16.8	11.2	11.2	8.4	11.2	8.4	17.9	9.5
Average	61.6	50.0	48.8	15.2	22.8	12.8	21.6	11.6	14.6	10.8	48.5	28.1
Sandy soil:												
Soil alone	56.0	30.8	14.0	5.6	16.8	11.2	19.6	11.2	14.0	14.0	24.1	14.6
Cottonseed meal	294.8	305.2	280.0	100.8	132.5	16.8	151.2	22.4	14.0	19.6	194.5	92.9
Manure	16.8	19.6	8.4	11.2	8.4	11.2	8.4	8.4	11.2	11.2	10.6	12.3
Timothy hay	11.2	8.4	11.2	8.4	16.8	11.2	14.0	11.2	11.2	89.6	12.9	25.8
Clover hay	39.2	39.2	19.6	11.2	14.0	14.0	11.2	8.4	16.8	14.0	20.1	17.4
Green timothy	58.8	47.6	33.6	16.8	14.0	8.4	11.2	8.4	5.6	5.6	24.6	17.8
Green clover	103.6	75.6	39.8	14.0	11.2	11.2	11.2	11.2	5.6	5.6	32.5	23.5
Average	97.2	75.2	56.8	24.0	30.5	12.0	32.4	11.6	17.2	22.8	45.6	29.2

Lime favors nitrification and at least in that indirect way indicates a retarded ammonification. Lime also increases the number of organisms, and should therefore tend to reduce the total of ammonia and nitrates in the presence of a limited supply of organic matter, because of greater nutritional demands by the increased number of organisms. When a large amount of nitrogenous organic matter is added perhaps this would not result. And since the ammonification process is the actual limiting factor under conditions which permit of nitrification, the increased basicity due to the use of lime evidently does have a retarding effect.

When averages are taken of all determinations and all treatments, there is no case on the humus soil (so-called because of its higher content of organic matter) where lime has not diminished the amount of ammonia produced. On the sandy soil there are two cases, with manure and with timothy hay, where the reverse is true, but the result would appear to be more nearly accidental than fundamental.

TABLE 2

Nitrates at each successive sampling

TREATMENT	FIRST SAMPLE, 2 WEEKS		SECOND SAMPLE, 5 WEEKS		THIRD SAMPLE, 10 WEEKS		FOURTH SAMPLE, 15 WEEKS		FIFTH SAMPLE, 22 WEEKS		AVERAGE	
	No lime	Lime	No lime	Lime	No lime	Lime	No lime	Lime	No lime	Lime	No lime	Lime
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Humus soil												
Soil alone.....	28.6	19.1	63.5	68.8	38.9	95.9	52.3	102.0	50.0	121.1	64.7	83.4
Cottonseed meal.......	33.0	45.7	98.3	243.2	214.8	309.0	302.4	289.9	324.0	316.0	194.5	240.8
Manure......	14.2	7.3	21.4	23.8	37.8	57.8	36.7	61.8	74.5	104.1	36.9	50.9
Timothy hay.	Tr.*	Tr.	Tr.	Tr.	Tr.	20.5	Tr.	35.5	22.8	67.4	4.5	24.7
Clover hay...	40.6	58.9	67.8	92.5	80.3	129.5	86.3	133.5	116.7	170.8	78.3	117.0
Green timothy.	45.6	51.5	100.4	83.9	180.5	125.0	141.1	93.8	181.4	121.4	129.8	94.9
Green clover..	69.4	78.1	109.7	122.0	234.1	319.1	181.5	168.1	284.6	201.0	175.8	177.6
Average....	33.1	37.2	65.9	90.6	112.3	150.9	114.3	127.8	150.6	165.9	95.2	112.8
Sandy soil:												
Soil alone.....	17.7	16.6	58.6	72.4	85.0	58.8	97.6	73.1	81.6	103.8	68.1	65.9
Cottonseed meal.......	9.4	7.3	112.2	138.3	167.9	229.4	267.6	400.2	312.4	457.4	173.9	246.5
Manure......	11.2	19.1	38.2	52.1	53.1	62.1	61.4	68.8	61.8	89.8	45.0	54.4
Timothy hay..	Tr.	Tr.	Tr.	Tr.	Tr.	14.8	Tr.	41.5	21.3	50.3	4.2	21.3
Clover hay...	11.5	15.1	63.6	97.1	83.5	69.5	90.7	122.0	123.1	152.4	74.5	91.2
Green timothy.	12.1	23.3	66.1	86.6	100.7	82.4	92.0	88.0	105.3	144.3	75.2	84.9
Green clover..	16.4	13.7	86.0	109.3	153.3	117.9	147.3	135.5	207.4	183.7	122.1	112.0
Average....	11.2	13.6	60.7	79.4	90.0	90.7	108.0	129.9	130.3	168.8	80.4	96.6

* Tr. = trace.

NITRIFICATION

For the determination of nitrates the phenoldisulfonic acid method as modified by Davis (1) was used. Calcium carbonate was employed to flocculate the soil and secure a clear filtrate. The results are given in table 2.

It is observed that the amount of nitrates increased in the untreated soils up to the last sampling.

The cottonseed meal, in accordance with its higher nitrogen content, gave a greater accumulation of nitrates on both soils than any other treatment. Here again, the sandy soil, though starting more slowly, finally ran higher than the better soil. On both soils, the greatest amount of nitrate was found at

the last sampling, the first two samples on the sandy soil showing less than the untreated soil. In most cases lime increased the nitrification of cottonseed meal.

The addition of stable manure caused a decrease in the amount of nitrates present in most cases, probably because of an increased number of organisms greater than the accompanying addition of easily nitrifiable material.

Timothy hay had the same effect as stable manure but to a much more marked degree. Little nitrifiable material was added in the timothy, but considerable energy material was provided, and the organisms used most of the nitrates for nutritional purposes. The nitrates began to show at about the same time on both soils but never ran nearly so high as on the untreated soils. Lime again stimulated nitrification. The green timothy in contrast to the dry, stimulated nitrification at once on both soils, and the greatest accumulation of nitrates was found at the last sampling and in the presence of lime.

Dry clover also caused a gradual stimulation of nitrification, the greatest effect being produced at the last sampling. The stimulation was usually greater also in the presence of lime. The green clover had a somewhat greater effect than did the dry, and maximum nitrification was induced sooner.

When averages of all samplings and all treatments are taken, the humus soil shows greater nitrification in the presence of lime in every case except one, and this is where green timothy was applied. There is very little difference with the green clover. When the sandy soil is considered the soil alone produces slightly less nitrates on the limed series. Every treatment except one, and in this case it is green clover, has shown greater nitrification in the presence of lime. Apparently the lime does not affect the nitrification of the green material as much as some of the dried materials. As is quite logical, the greatest amount of nitrates is found at the last sampling, while the greatest amount of ammonia is usually found at the first sampling.

A summary of the nitrate and ammonia determinations is given in table 3.

The table shows the largest combined production of nitrates and ammonia where cottonseed meal was applied, followed in order by green clover, green timothy, horse manure, and dry timothy, the latter two producing considerably less than the soil alone. The general effect of the lime was to decrease the total of nitrates and ammonia found, especially where there was any large production.

ACIDITY RESULTS

The lime requirements on the soils differently treated are given in table 4. The determinations were made according to the modified Tacke method previously described (5). The acid soil was brought into contact with pure calcium carbonate, and the aeration and shaking continued for 10 hours before titrations were made. The double-titration was performed, with methyl-orange and phenolphthalein as indicators.

TABLE 3

Nitrogen summary; summary of ammonia and nitrates

TREATMENT	FIRST SAMPLE 2 WEEKS		SECOND SAMPLE 5 WEEKS		THIRD SAMPLE 10 WEEKS		FOURTH SAMPLE 15 WEEKS		FIFTH SAMPLE 22 WEEKS		AVERAGE			
	No lime	Lime	No lime	Lime	No lime	Lime	No lime	Lime	No lime	Lime	No lime		Lime	
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	Minus soil *p.p.m.*	*p.p.m.*	Minus soil *p.p.m.*
Humus soil:														
Soil alone	45.5	33.1	74.7	77.3	55.7	107.2	63.6	131.1	61.2	134.5	60.1		96.6	
Cottonseed meal	335.5	331.3	367.1	304.8	312.9	331.4	338.2	309.5	346.1	330.0	339.9	279.8	321.4	224.8
Manure	22.6	13.0	32.6	33.3	49.3	69.0	50.8	73.0	88.5	115.3	48.7	−11.4*	60.7	−35.9*
Timothy hay	5.6	8.4	11.2	11.2	16.8	31.6	11.2	41.1	34.0	78.6	15.7	−44.4*	34.2	−62.4*
Clover hay	79.2	70.1	76.5	98.1	91.5	140.6	91.9	144.7	127.7	170.8	93.4	33.3	124.8	28.2
Green timothy	90.4	62.7	114.4	89.5	197.3	136.1	152.2	105.0	192.6	132.6	149.4	89.3	105.2	8.6
Green clover	103.1	92.1	125.5	127.6	250.9	330.3	192.7	176.5	295.8	209.4	193.8	133.7	187.2	90.6
Average											128.7	134.0	132.9	88.1
Sandy soil:														
Soil alone	73.8	37.4	72.6	78.0	101.8	70.0	117.2	84.8	95.6	117.8	92.2		77.6	
Cottonseed meal	404.2	312.5	392.2	239.1	300.4	246.2	418.8	422.6	326.4	477.0	368.4	276.2	339.5	261.9
Manure	28.0	38.7	46.6	63.4	61.5	73.7	69.5	57.2	72.6	101.0	55.6	−36.6*	66.8	−10.8*
Timothy hay	11.2	8.4	11.2	8.4	16.8	26.1	14.0	52.7	32.5	139.9	17.1	−75.1*	47.1	−3.0*
Clover hay	50.8	54.3	143.2	107.3	97.6	83.5	101.9	130.4	139.8	171.4	106.7	14.5	109.4	31.8
Green timothy	70.9	70.9	99.7	103.4	114.7	90.8	103.2	101.4	110.9	149.9	99.9	7.7	103.3	25.7
Green clover	114.0	89.3	116.8	123.3	164.5	129.1	158.5	146.7	212.6	189.3	153.3	61.1	135.5	57.9
Average											127.6	89.9	125.6	94.3

* These are omitted in taking final averages.

There is little to be said in regard to the effect of the various treatments upon the lime requirement of the soils. The general tendency has been to

TABLE 4

Lime requirement of the variously treated soils in tons per 2,000,000 pounds soil

TREATMENT	FIRST SAMPLE, 2 WEEKS	SECOND SAMPLE, 5 WEEKS	THIRD SAMPLE, 10 WEEKS	FOURTH SAMPLE, 15 WEEKS	FIFTH SAMPLE, 22 WEEKS	MORE OR LESS THAN THE SOIL ALONE				
						First sample	Second sample	Third sample	Fourth sample	Fifth sample
	tons	*tons*	*tons*	*tons*	*tons*	*tons*	*tons*	*tons*	*tons*	*tons*
Humus soil:										
Soil alone	3.90	4.20	3.85	3.80	3.80					
Cottonseed meal	3.65	3.65	4.45	4.25	4.55	−0.25	−0.55	+0.60	+0.45	+0.75
Manure	3.80	4.25	3.60	3.40	3.80	−0.10	+0.05	−0.25	−0.40	+0.00
Mature timothy	4.05	4.15	3.55	3.35	3.55	+0.15	+0.05	−0.30	−0.45	−0.25
Mature clover	3.35	4.15	3.65	3.25	3.95	−0.05	−0.05	−0.20	−0.55	+0.15
Green timothy	4.10	4.45	3.70	3.65	3.95	+0.20	+0.25	−0.15	−0.15	+0.15
Green clover	3.6	4.00	3.25	3.20	3.85	−0.20	−0.20	−0.60	−0.60	+0.05
Sandy soil:										
Soil alone	3.20	2.60	2.35	2.40	2.35					
Cottonseed meal	1.70	2.15	2.15	2.50	2.45	−0.50	−0.45	−0.20	+0.10	+0.10
Manure	2.20	2.35	2.10	2.65	1.75	0.00	−0.25	−0.25	+0.25	−0.60
Mature timothy	2.20	2.30	1.80	2.05	1.75	0.00	−0.30	−0.55	−0.35	−0.60
Mature clover	2.15	2.30	1.90	1.80	1.75	−0.05	−0.30	−0.45	−0.60	−0.60
Green timothy	2.55	2.65	2.25	2.30	2.00	+0.35	+0.05	−0.10	−0.10	−0.35
Green clover	1.70	2.65	1.90	1.90	1.85	−0.50	+0.05	−0.45	−0.50	−0.50

TABLE 5

Difference of ammonia and nitrates on unlimed soils compared with effect of treatment on lime requirement

Humus soil:					
Ammonia (p.p.m.)	302.4	268.8	98.0	86.8	32.0
Nitrates (p.p.m.)	33.0	98.3	214.8	302.4	324.0
Difference (p.p.m.)	+269.4	+170.5	−116.8	−215.6	−292.0
Difference in lime requirement (tons)	−0.25	−0.55	+0.60	+0.45	+0.75
Sandy soil:					
Ammonia (p.p.m.)	394.8	280.0	132.5	151.2	14.0
Nitrates (p.p.m.)	9.4	112.2	167.9	267.6	312.4
Difference (p.p.m.)	+385.4	+168.8	−35.4	−116.4	−298.4
Difference in lime requirement (tons)	−0.50	−45.0	−0.20	+0.10	+0.10

reduce rather than to increase it. A large production of ammonia reduces the lime requirement, and, quite logically, when nitrification occurred the opposite effect resulted.

Table 5 brings out this point when the cottonseed meal treatment is studied, in comparing the effect of ammonification and nitrification upon the decrease or increase of the lime requirement of the treated soil over the untreated.

This table shows that though there is not a close correlation between the difference of ammonia and nitric acid produced on the soils treated with cottonseed meal, and the effect upon the lime requirement, the tendency is for the soil to show a greater or smaller lime requirement according as there is more or less of the nitrogen present in the basic or acid form. None of the other treatments contain sufficient nitrogen to make the comparison significant.

TABLE 6

Residual carbonates on treated soils; expressed in tons per acre

TREATMENT	FIRST SAMPLE, 2 WEEKS	SECOND SAMPLE, 5 WEEKS	THIRD SAMPLE, 10 WEEKS	FOURTH SAMPLE, 15 WEEKS	FIFTH SAMPLE, 22 WEEKS	MORE OR LESS THAN SOIL UNTREATED				
						First sample	Second sample	Third sample	Fourth sample	Fifth sample
	tons	*tons*	*tons*	*tons*	*tons*	*tons*	*tons*	*tons*	*tons*	*tons*
Humus soil:										
Soil alone	3.40	2.55	2.00	1.95	1.35					
Cottonseed meal	4.95	2.55	1.20	1.25	0.55	+1.55	+0.00	−0.80	−0.70	−0.80
Manure	4.10	2.85	2.45	1.90	2.05	+0.70	+0.30	+0.45	−0.05	+0.70
Dry timothy	4.35	2.90	2.35	2.10	1.90	+0.95	+0.35	+0.35	+0.15	+0.55
Dry clover	4.15	3.05	2.30	2.10	2.15	+0.75	+0.50	+0.30	+0.15	+0.80
Green timothy	4.05	3.20	2.50	2.30	2.15	+0.65	+0.65	+0.50	+0.35	+0.80
Green clover	4.20	3.00	2.95	2.50	2.45	+0.80	+0.45	+0.95	+0.55	+1.10
Sandy soil:										
Soil alone	2.80	2.55	2.45	2.55	2.40					
Cottonseed meal	3.90	2.35	1.70	1.20	0.85	+1.10	−0.20	−0.75	−1.35	−1.55
Manure	2.95	2.70	2.65	2.60	2.40	+0.15	+0.15	+0.20	+0.05	+0.00
Dry timothy	3.25	2.75	2.45	2.60	2.35	+0.45	+0.20	+0.00	+0.05	−0.05
Dry clover	3.30	3.00	2.75	2.90	2.50	+0.50	+0.45	+0.30	+0.35	+0.10
Green timothy	2.80	2.55	2.50	2.40	2.30	+0.00	+0.00	+0.05	−0.15	−0.10
Green clover	4.25	3.30	3.00	3.00	2.85	+0.45	+0.75	+0.55	+0.45	+0.45

RESIDUAL CARBONATES

The residual carbonates were determined by decomposing the remaining limestone with dilute acid, and titrating the carbon dioxide liberated, in the same way as the titration was made in the lime-requirement determinations. The results are given in table 6.

Lime was applied at the rate of 7 tons on the more acid soil and 6 tons on the other soil, in the form of the precipitated carbonate. As was intended a sufficient excess was added so that nitrification did not exhaust it.

The data show that in most cases the organic materials have tended to protect the lime applied to the soil. The notable exception is the cottonseed meal, which on account of the large production of nitric acid, has used up the

carbonates nearly completely. All of the treatments helped to save limestone until nitrification occurred, as noted by the fact that with but three exceptions minus quantities do not appear until the last two samplings.

SOLUBLE NON-PROTEIN NITROGEN

The method employed in this study was in general that used by Potter and Snyder (4). The soil was extracted with 1 per cent hydrochloric acid, in both the limed and the unlimed series. The nitrate nitrogen and the ammonia nitrogen were distilled off by the Devarda reduction method. The residue from this reduction was then treated with sulfuric acid and the total nitrogen determined in the usual way. This latter gave the unknown soluble non-protein nitrogen of the acid extract.

The acid-extracted soil was next extracted by shaking 2 hours with 1.75 per cent sodium hydroxide, and the extract clarified by centrifuging for 5 minutes at 30,000 revolutions per minute. The extract was then neutralized with sulfuric acid, and acidified with tri-chlor-acetic acid sufficiently to give $2\frac{1}{2}$ per cent of the latter. The precipitate was then filtered off and another aliquot of the filtrate taken for determination of the nitrogen by the micro-method.

TABLE 7

Soluble non-protein nitrogen in humus soil after 5 weeks

TREATMENT	UNKNOWN NON-PROTEIN NITROGEN				TOTAL UNKNOWN NON-PROTEIN NITROGEN	
	In HCl extract		In alkaline extract			
	No lime	Lime	No lime	Lime	No lime	Lime
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Soil alone	23.33	26.00	245.5	246.5	268.83	272.50
Cottonseed meal	195.99	44.66	310.5	287.5	506.49	355.16
Manure	32.66	11.33	246.5	218.0	278.16	229.33
Timothy	28.66	29.99	232.0	253.0	260.66	282.99
Clover	23.33	26.00	260.5	244.0	283.83	270.00
Green timothy	35.33	30.00	266.0	253.0	301.33	283.00
Green clover	28.66	19.60	277.3	253.3	305.96	272.90

Soluble non-protein materials should probably be the largest in amount when decomposition is the most active. But the question is, do these compounds, many of which are doubtless of a peptide character, tend to accumulate in soils in appreciable amounts, or do ammonification and nitrification proceed at once when the decomposition has started. In other words, should the soluble nitrogen be found primarily in the form of ammonia and nitrates or also in more complex forms? Previous study has shown that plants are capable of using more complex forms of nitrogen than nitrates and ammonia, and if they occur to any extent in ordinary soils, there may be conditions when such complex compounds function as direct sources of plant-food.

The results show in every case but one (timothy) that the application of lime has diminished the total unknown soluble non-protein nitrogen. The nitrates and ammonia, though soluble non-protein nitrogen, are not included in these data. A reference to table 3 shows that this is the same general tendency as observed in the production of ammonia. There is one noticeable fact, and that is that none of the organic treatments have as marked an effect upon the amount of unknown soluble non-protein nitrogen as they have on the nitrates and ammonia. This indicates, as do also the data of Potter and Snyder (4), that in the decomposition of proteins of the soil the degradation products undergo rather rapidly a complete change to the simpler state of ammonia. and nitrate. Except in case of the more resistant forms, possibly polypeptides of some degree of complexity, the products apparently do not accumulate to a large extent, and the nitrogen of the soil must exist mostly as the more complex and resistant forms or else as the simplest possible products of decomposition. Ordinarily, of course, nitrates and ammonia are removed from the soil almost as rapidly as produced, and therefore they are not found in large amounts at any one time. Hence the soluble non-proteins such as are found in this study are probably present at any definite time in perhaps five or even ten times the amount of ammonia and nitrates present.

Another question to consider is the possible effect of such compounds on the reaction of the soil. Though perhaps capable of reaction as either acids or bases, they are not found in sufficient quantity to exert a marked effect upon soil reaction. Such materials and others, however, doubtless exercise a buffering effect and help to reduce the hydrogen-ion concentration to some extent.

GENERAL DISCUSSION

This experiment was continued for 159 days, or about 22 weeks. It is not presumed that there would be no change after this time, but rather that such changes as occurred previous to this would determine whatever effects were to be produced by the different treatments on the activity of soil organisms or the reaction of the soil.

The materials used contained the following percentages of nitrogen: dry timothy, 0.693; manure, 1.24; green timothy, 1.28; dry clover, 2.30; green clover, 2.82; and cottonseed meal, 6.96 per cent. The poorer soil contained 0.116, and the better soil about twice as much, or 0.238 per cent, of nitrogen. The amounts of nitrogen found as ammonia and nitrates were for the most part in the same order as the percentages of nitrogen contained in the materials used.

No definite conclusions may be drawn from a limited study, but in general it seems that the essential soil organisms are active in soils of at least moderately strong acidity. The data indicate also that the decay of organic materials under aerobic conditions does not produce an appreciable acidity except where nitric acid is formed in nitrification.

SUMMARY

1. The lime requirement of neither soil was increased by the organic treatments except in those cases where there was a large production of nitric acid.

2. Ammonification is apparently greater in the absence of lime, partly perhaps because of the fact that nitrifying organisms have been less active.

3. Lime has generally stimulated nitrification.

4. The sum of ammonia and nitrates is usually greater on the unlimed soil when treated with nitrogenous organic materials. This is doubtless partly due to the increased number of organisms in the presence of lime and the consequent greater consumption of nitrates and ammonia by the organisms.

5. When nitrogenous sources of energy such as horse manure and timothy hay were supplied, nitrifiction and ammonifiction were reduced below that of the untreated soil.

6. The green materials were somewhat more readily attacked than the dried materials. There was greater production of ammonia and nitrates partly however because of the fact that these materials were richer in nitrogen than the mature plants.

7. The soluble unknown non-protein nitrogen determined at the second sampling on the more fertile soil, when the activity of the organisms was nearly at the maximum, showed little effect due to the various organic treatments. The cottonseed meal was the only treatment which gave any large increase over the untreated soil.

8. In all cases but one, the unlimed treatments gave a higher non-protein nitrogen content than the limed.

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THE EFFECT OF ORGANIC MATTER ON SOIL REACTION. II[1]

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INTRODUCTION

A study on the effect of organic matter on soil reaction was undertaken as a part of an extended investigation of soil acidity. For a description of the background of the experiments here reported, experimental methods, etc., the reader is referred to the preceding study (5), also to a former study of the same problem (4).

In this series of treatments the organic materials were applied at the same rates as before (10 tons) (4) except where dried blood and straw were mixed and then blood was used at the rate of 10 tons, with 5 and 10 tons of straw. Precipitated carbonate of lime was added to the limed treatments at the uniform rate of 5 tons per acre. The materials used were soybean hay, green rape, oat straw, green soybean hay (pods removed), dried blood and a mixture of blood and oat straw, all in both the limed and the unlimed series. The green materials were dried, as were also the other materials, and ground as finely as was convenient before adding to the soil. The soil used in this study was an acid silt loam taken from the West Virginia Agricultural Experiment Station farm, rather heavy and compact, and poor in organic matter.

The total period of incubation was 125 days, samplings being made at intervals of 2, 5, 10 and 18 weeks, respectively. In addition to the determinations made in the study of the previous series, hydrogen-ion determinations were made upon all treatments.

AMMONIFICATION

The aeration method was again used for ammonia. The results are shown in table 1, expressed as parts of nitrogen per million of soil.

Only the blood possessed a high nitrogen content and therefore it is the only material which caused a large production of ammonia.

[1] This paper is the second part of a thesis presented to the graduate faculty of the Iowa State College of Agriculture in partial fulfillment of the requirements for the degree of Doctor of Philosophy. It is also the second paper published on this study, the former (4) having appeared in 1919. A portion of the work here reported was completed at the Iowa Agricultural Experiment Station, and the remainder was conducted in consultation with Prof. R. M. Salter at West Virginia University. Acknowledgments are extended to Dr. P. E. Brown, of Iowa State College, and also to Professor Salter, for helpful suggestions in planning and interpreting the work.

Lime produced no marked effect in the ammonification of any of the materials until the third sampling, when it caused an appreciable reduction, which was still very evident at the last sampling. This may have been due to two causes. The lime may have caused greater numbers of organisms to grow, which in turn caused a greater consumption of ammonia. The principal cause, no doubt, was that lime permitted greater nitrification, and most of the ammonia had been changed over to nitrate. The data show that this had occurred.

The oat straw depressed ammonification just as it did nitrification, in most cases below that of the untreated soil; this would indicate that it was a suitable source of energy for bacterial activity.

Green soybeans likewise depressed ammonification below that of the soybean hay but partly because of the fact that their nitrogen content was lower.

TABLE 1

Amount of ammonia at the end of each period

TREATMENT	FIRST SAMPLE, 2 WEEKS		SECOND SAMPLE, 5 WEEKS		THIRD SAMPLE, 10 WEEKS		FOURTH SAMPLE, 18 WEEKS		AVERAGES	
	No lime	Lime	No lime	Lime	No lime	Lime	No lime	Lime	No lime	Lime
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Silt loam soil:										
Soil alone	3.6	50.0	32.0	34.0	60.0	5.6	16.0	12.0	36.0	2.54
Soybean hay	94.0	106.0	107.0	106.0	92.4	8.7	36.0	8.0	82.4	57.2
Green rape	182.0	168.0	178.0	132.0	36.4	8.7	64.0	4.0	115.1	78.2
Green soybeans	48.0	63.0	40.0	56.0	16.9	22.2	12.0	8.0	29.2	38.5
Oat straw	52.0	20.0	24.0	16.0	13.8	5.6	10.4	8.0	25.1	12.4
Blood	342.0	282.0	566.0	425.0	546.0	361.2	328.0	54.0	445.5	287.3
Blood and 5 tons of straw	242.0	316.0	424.0	400.0	336.2	43.2	440.0	48.0	360.5	201.8
Blood and 10 tons of straw	226.0	300.0	396.0	306.0	288.4	26.8	366.0	32.0	319.1	166.2
Averages	169.4	180.0	247.9	209.7	190.1	68.1	179.5	23.1	196.7	120.2

Green rape, on the other hand, stimulated ammonification next to the dried blood. However, it contained a little less nitrogen than the soybean hay, though more than the green soybeans.

Straw mixed with blood had little consistent effect upon ammonification. However, the ammonia produced by the combined application of blood and straw was seldom greater and often less than that produced from the blood alone.

There were individual cases where the limed treatments produced more ammonia than the unlimed, but when averages of all treatments (omitting the soil alone) and of all samples, were taken, the unlimed treatments have produced a greater quantity of ammonia. The difference is quite marked at later samplings when nitrification is well under way.

The data show that the accumulation of nitrates has increased at each successive sampling with all treatments, as well as with the untreated soil,

in both the limed and the unlimed series. In general, there has been greater nitrification in the presence of lime. This is more noticeable after the first sampling and with the nitrogen rich materials. Lime apparently had the opposite effect where oat straw was used. Straw used with blood retarded nitrification at first but later there was little or no retardation. The maximum amount of nitrates occurred at the last sampling in most cases.

Apparently the green soybeans began to nitrify more quickly than did the soybean hay. Green rape likewise at once stimulated nitrification to an appreciable extent.

NITRIFICATION

Nitrates were determined by the colorimetric method as before. The results are shown in table 2.

TABLE 2

Nitrates at each successive sampling

TREATMENT	FIRST SAMPLE, 2 WEEKS		SECOND SAMPLE, 5 WEEKS		THIRD SAMPLE, 10 WEEKS		FOURTH SAMPLE, 18 WEEKS		AVERAGES	
	No lime	Lime	No lime	Lime	No lime	Lime	No lime	Lime	No lime	Lime
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Silt loam soil:										
Soil alone	19.6	23.0	24.8	33.3	38.4	54.6	65.3	113.8	37.0	56.2
Soybean hay	25.9	25.7	28.8	37.9	71.8	181.1	122.6	195.3	62.3	110.0
Green rape	76.1	96.6	79.0	75.1	120.5	234.1	260.3	188.7	133.9	148.6
Green soybeans	42.5	27.2	388.5	39.7	83.5	108.2	175.7	109.0	172.5	71.0
Oat straw	19.8	Tr.*	19.6	4.6	26.3	16.2	38.6	38.9	26.1	15.4
Dried blood	14.2	9.1	24.5	40.7	149.1	485.5	353.3	611.1	135.3	286.6
Blood and 5 tons of straw	8.6	Tr.	17.3	37.1	160.3	280.9	332.7	640.0	129.8	264.5
Blood and 10 tons of straw	Tr.	Tr.	59.1	59.8	156.9	492.5	413.0	575.4	157.2	281.9
Average	26.7	22.7	88.2	42.1	109.8	271.5	242.3	336.9	116.7	168.3

* Tr. = trace.

Nitrification apparently scarcely occurred in the presence of oat straw until the third and fourth samplings. In no case was there as much nitrate as on the untreated soil.

Nitrification was slow in starting when blood and straw were mixed but by the end of 10 weeks there was an appreciable accumulation of nitrates on the treated soils over the untreated. Apparently the addition of straw had no marked effect upon the nitrification of dried blood.

When averages of all treatments and all samplings are taken (omitting the untreated soil) it is observed that nitrification was slow in starting where straw and blood and mixtures of the two were used, but the blood-straw mixtures finally ran high. Lime in these cases seems to have retarded the beginning of the nitrifying process, but perhaps more organisms were present where lime was added and they were consuming such nitrates as were produced.

The nitrogen summary shown in table 3 indicates that the average total of nitrates and ammonia has been greatest in most cases for the treated soils, when not limed, but that the reverse is true for the untreated soil. Whether the difference may be due to numbers of organisms and the consequent utilization of part of the nitrogen changed on treated limed soils, cannot be stated, though it seems probable. Experience has shown that in nearly every case a carbohydrate material such as straw which is poor in nitrogen, has given a decrease in ammonia and nitrates over the soil alone, either limed or unlimed. Since the ammonia and nitrate forms of nitrogen are by-products of the attempt of the organism to secure sufficient energy, this is to be expected.

TABLE 3

Nitrogen summary, nitrates and ammonia

TREATMENT	FIRST SAMPLE, 2 WEEKS		SECOND SAMPLE, 5 WEEKS		THIRD SAMPLE, 10 WEEKS		FOURTH SAMPLE, 18 WEEKS		AVERAGE			
	No lime	Lime	No lime	Lime	No lime	Lime	No lime	Lime	No lime		Lime	
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	Minus soil	*p.p.m.*	Minus soil
Silt loam soil:												
Soil alone	55.6	73.0	57.3	67.3	98.4	59.9	81.3	125.8	73.1	*p.p.m.*	81.5	*p.p.m.*
Soybean hay	120.0	131.8	130.8	144.0	164.2	189.8	158.6	203.3	143.4	70.3	167.2	85.7
Green rape	258.1	264.6	257.0	207.1	156.9	242.8	324.3	192.7	249.1	176.0	226.8	145.3
Green soybeans	90.5	95.2	78.5	95.7	100.4	130.4	187.7	117.0	114.3	−41.2	109.8	28.3
Oat straw	71.8	20.0	43.6	20.6	40.1	23.8	49.1	46.9	51.2	−21.9	27.8	−53.7
Dried blood	356.2	291.1	590.6	492.7	745.1	846.7	681.1	665.1	580.8	507.7	548.9	467.4
Blood and 5 tons of straw	250.6	316.0	441.8	437.3	496.5	424.1	777.7	687.9	491.7	418.6	475.8	394.3
Blood and 10 tons of straw	266.0	300.0	455.0	365.8	445.3	519.2	779.0	607.4	477.2	404.1	488.1	366.6
Average	196.1	202.7	336.1	251.8	299.9	339.6	421.8	360.0	301.1		286.3	

LIME REQUIREMENT

The data show that in nearly every case the lime requirement was less when organic matter was added to the soil (table 4). The greatest effect was usually at the first sampling. This was especially marked with the dried blood which produced large amounts of ammonia. Next to blood, soybean hay produced the greatest effect; green rape was next and oat straw last. Thus it seems that nitrogenous materials, by their production of ammonia and perhaps by other reactions, reduce the lime requirement of soils. The effect has been more marked and consistent on this rather heavy soil than on the lighter soils previously studied. Carbohydrate materials have much smaller effects.

It is shown also that the limed soils have a capacity for decomposing limestone, even after 18 weeks' standing with an excess of lime. This would indicate that acid soils react with carbonate of lime beyond the neutral point, or that for lack of sufficiently intimate contact, all the acids have not yet been neutralized. There is perhaps no such thing as completion of the reaction. There are doubtless always soluble acids or acid salts capable of decomposing the carbonate.

TABLE 4

Lime requirement of variously treated soils (tons per 2,000,000 pounds)

	FIRST SAMPLE, 2 WEEKS	SECOND SAMPLE, 5 WEEKS	THIRD SAMPLE, 10 WEEKS	FOURTH SAMPLE, 18 WEEKS	MORE OR LESS THAN SOIL ALONE			
					First sample	Second sample	Third sample	Fourth sample
	tons	*tons*	*tons*	*tons*	*tons*	*tons*	*tons*	*tons*
Clay soil:								
Soil alone	3.35	2.95	3.10	3.10				
Soil limed	0.95	0.55	0.45	0.65				
Soybean hay	2.00	2.60	2.60	3.10	−1.35	−0.35	−0.50	+0.00
Limed	0.35	0.95	0.60	0.80	−0.60	+0.40	+0.15	+0.15
Green rape	2.10	2.50	2.65	3.20	−1.25	−0.45	−0.45	+0.10
Limed	0.60	0.80	0.55	0.90	−0.35	+0.25	+0.10	+0.25
Green soybeans	1.85	2.85	2.65	3.00	−1.50	−0.10	−0.45	−0.10
Limed	0.45	0.95	0.55	0.60	−0.50	+0.40	+0.10	−0.05
Oat straw	2.65	2.60	2.65	2.85	−0.70	−0.35	−0.45	−0.25
Limed	0.45	0.75	0.75	0.65	−0.50	+0.30	+0.30	+0.00
Blood	2.00	1.80	2.00	2.95	−1.35	−1.15	−1.10	−0.15
Limed	0.35	0.90	0.65	1.35	−0.60	+0.35	+0.20	+0.70
Blood and 5 tons of straw	1.85	2.65	1.90	3.05	−0.50	−0.30	−1.20	−0.05
Limed	0.35	1.05	0.80	1.50	−0.60	+0.50	+0.35	+0.85
Blood and 10 tons of straw	1.70	2.20	2.05	3.00	−0.65	−0.75	−1.05	−0.10
Limed	0.40	1.20	0.90	1.40	−0.55	+0.65	+0.45	+0.75

It is worthy of note, too, that the organic treatments seem to have increased the capacity of the soil to react with lime, when they were used alone.

There is a rather close correlation between changes in soil reaction, and the nitrogen changes as shown by table 5. This is especially noticeable on the blood treatments where there is sufficient nitrogen added to produce a measurable effect upon the reaction.

These data show a close correlation between the excess of ammonia over nitrates and the true acidity, or pH values of the soils, and would signify that the bacteriological changes which were occurring were affecting the soil reaction to an appreciable extent.

The same thing is shown in table 6 on all treatments, considering the summarized effects as before.

TABLE 5

Difference of ammonia and nitrates on unlimed soils compared with the effect of the treatment on soil reactions

SILT LOAM SOIL	FIRST SAMPLE	SECOND SAMPLE	THIRD SAMPLE	FOURTH SAMPLE
Blood treatment only				
Ammonia (p.p.m.)	270.0	462.0	390.0	378.0
Nitrates (p.p.m.)	7.8	33.8	155.4	366.3
Excess (NH_3) (p.p.m.)	+262.2	+428.0	+234.6	+ 11.7
pH on blood	6.33	7.00	6.46	5.41
pH increase over untreated soil	+1.62	+2.12	+1.68	+0.54

TABLE 6

Nitrogen changes and the effect on soil reaction summarized

ALL TREATMENTS NO LIME	FIRST SAMPLE	SECOND SAMPLE	THIRD SAMPLE	FOURTH SAMPLE
Ammonia (p.p.m.)	169.4	247.9	190.1	179.5
Nitrates (p.p.m.)	26.7	88.2	109.8	242.3
Difference (p.p.m.)	+142.7	+159.7	+80.3	−62.8
pH values	6.01	6.23	5.97	5.17
pH increase over untreated soil	+1.10	+1.35	+1.19	+0.29

TABLE 7

Residual carbonates on treated soils at the various samplings, expressed as tons per acre

	FIRST SAMPLE	SECOND SAMPLE	THIRD SAMPLE	FOURTH SAMPLE	MORE OR LESS THAN SOIL ALONE			
					First sample	Second sample	Third sample	Fourth sample
	tons	*tons*	*tons*	*tons*	*tons*	*tons*	*tons*	*tons*
Clay soil:								
Soil alone	2.35	2.20	1.40	0.90				
Soybean hay	3.15	2.40	1.05	1.05	+0.60	+0.20	−0.35	+0.15
Green rape	3.15	2.10	1.20	0.45	+0.60	−0.10	−0.20	−0.45
Green soybeans	3.25	2.00	1.45	1.00	+0.70	−0.20	+0.05	+0.10
Oat straw	3.20	1.85	1.40	0.95	+0.65	−0.35	+0.00	+0.05
Blood	3.15	3.05	0.60	0.00	+0.60	+0.85	−0.80	−0.90
Blood and 5 tons of straw	3.70	3.20	1.05	0.10	+1.15	+1.00	−0.35	−0.90
Blood and 10 tons of straw	3.60	3.20	0.70	0.00	+1.05	+1.00	−0.70	−0.90

RESIDUAL CARBONATES

The data show that the organic matter protected the carbonates until there was considerable nitrification. All organic treatments caused a marked saving of carbonates at the first sampling. At the last sampling those treatments

where there was much nitrogen to produce nitric acid, nearly or completely exhausted the carbonates present. Even the untreated soil reacted slowly and continually and would perhaps have used up all the limestone after sufficient time, even though there was no leaching.

These data would indicate that excessive nitrification might become a positive factor in contributing to soil acidity. However, nitrates, being soluble, will not accumulate and in the process of leaching basic material is permanently removed from the soil.

HYDROGEN-ION CONCENTRATION

The hydrogen-ion concentration was determined at each sampling on all of the treatments with the hydrogen electrode apparatus.

TABLE 8

Hydrogen-ion concentration

	FIRST SAMPLE	SECOND SAMPLE	THIRD SAMPLE	FOURTH SAMPLE	MORE OR LESS THAN SOIL ALONE			
					First sample	Second sample	Third sample	Fourth sample
	pH	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
Clay soil	4.91	4.88	4.78	4.88				
Soil alone:								
Soil and 5 tons of lime	7.62	7.72	7.65	7.60				
Soybeans and straw	6.03	5.89	5.50	5.02	+1.12	+1.01	+0.72	+0.14
Soybeans and lime	7.74	7.64	7.41	7.51	+0.12	−0.08	−0.24	−0.03
Green rape	6.03	6.05	6.17	4.78	+1.12	+1.17	+1.39	−0.10
Green rape and lime	7.66	7.65	7.42	7.53	+0.04	−0.07	−0.23	−0.07
Green soybeans	5.81	5.58	5.34	5.17	+0.90	+0.70	+0.56	+0.29
Green soybeans and lime	7.74	7.64	7.60	7.74	+0.12	−0.08	−0.05	+0.14
Oat straw	5.21	5.07	5.41	5.00	+0.30	+0.19	+0.63	+0.12
Oat straw and lime	7.48	7.60	7.66	7.71	−0.14	−0.12	+0.01	+0.11
Blood	6.48	7.17	6.55	5.43	+1.57	+2.29	+1.77	+0.55
Blood and lime	7.71	7.91	7.22	7.60	+0.09	+0.19	−0.43	+0.00
Blood and 5 tons of straw	6.28	7.10	6.58	5.38	+1.37	+2.22	+1.80	+0.50
Blood, 5 tons of straw and lime	7.74	7.76	7.34	7.54	+0.12	+0.04	−0.31	−0.06
Blood and 10 tons of straw	6.24	6.74	6.24	5.44	+1.33	+1.86	+1.46	+0.56
Blood, 10 tons of straw and lime	7.65	7.76	7.36	7.61	+0.03	+0.04	−0.29	+0.01

The lime requirement according to the Tacke method was a little more than 3 tons. To take care of acids which might be produced in the decomposition of organic material, an excess of 2 tons was used. The data (table 8) show that this was sufficient to give a slightly alkaline soil either with or without organic treatment (the smaller the pH value the more acid the soil). Every organic treatment without lime diminished the true acidity of the soil, the highly nitrogenous materials most, as was true also of the lime requirement. The oat straw had the least effect. In the presence of lime, however, the organic treatments had a rather slight effect in reducing the hydrogen-ion concentration at first, and by the third sampling the effect was the reverse in

nearly every case, though again the increase in hydrogen-ion concentrations was not large. By the fourth sampling the effects were quite erratic. In nearly every case where lime was not used, however, the organic treatments reduced the acidity somewhat.

GENERAL DISCUSSION

The materials used in this study were such as are common crop residues or fertilizers. The nitrogen content was: oat straw, 1.05; green soybeans, 2.41; green rape, 3.43; soybean hay, 6.63; and dried blood, 13.93 per cent. The 5-ton application of limestone proved to be scarcely enough to take care of the natural soil acidity plus that produced in nitrification as shown by the data.

The lime requirement shown by the Tacke method on this soil was about 3 tons. Shaking and aeration was continued for only 5 hours, however, in this and the remaining work, partly for convenience and partly because of the fact that a limited amount of work had shown that the lime requirement indicated by a 5-hour run was sufficient. When that quantity of lime was added to the soil and allowed to stand for a short time with optimum moisture conditions, a practically neutral reaction was shown by hydrogen-ion determinations.

The results of the effect of carbohydrate materials upon nitrification have a practical bearing which is worthy of consideration. Experience has shown that the plowing under of green manures such as rye, the heavy use of straw, and other refuse, often cause disappointing yields from the crop immediately following. This may result not alone because the crop has exhausted the water supply previous to plowing under, but oftentimes no doubt, because such materials have furnished the soil organisms with easily available sources of energy, and nitrification does not proceed rapidly enough to supply the crop with nitrates. Thus the immediate crop suffers nitrogen starvation, though perhaps later crops might be much benefited.

SUMMARY

1. Oat straw again reduced nitrification and ammonification below that of the untreated soil.

2. A mixture of straw and blood reduced the total nitrogen found in the form of ammonia and nitrates below that of the blood treatment alone. Ten tons of straw with the blood caused a somewhat greater reduction than the 5-ton application.

3. All the treatments reduced the lime requirement indicated by the Tacke method, until nitrification had taken place.

4. Lime-requirement determinations of the limed soils showed that the treated soils were always capable of reaction with more lime, though an excess of 2 tons of limestone had been applied. This shows that the soils

contain acids which are very slowly reactive, and perhaps they will react with limestone beyond their neutral point.

5. The residual carbonates, where blood was applied, were completely exhausted at the last sampling.

6. The hydrogen-ion determinations show that in practically every case the organic treatments reduced the true acidity. In some cases, on the contrary, both lime and organic treatments did not give as alkaline a soil as did the lime alone.

7. Changes in soil reaction especially on the blood-treated soils, follow very closely the deficit or excess of ammonia over nitric nitrogen, indicating that these processes may become factors influencing the production of acid soils.

BUFFERING IN SOILS

Practically all soils possess perhaps some degree of buffering, that is, they are able to react with either base or alkali to a certain extent, without very

TABLE 9

Table showing treatments and the hydrogen-ion concentration increments corresponding

SOIL	ALONE	1000 POUNDS $Ca(OH)_2$	2000 POUNDS $Ca(OH)_2$	4000 POUNDS $Ca(OH)_2$	8000 POUNDS $Ca(OH)_2$	16,000 POUNDS $Ca(OH)_2$	32,000 POUNDS $Ca(OH)_2$
	pH	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
A. Muck..............	4.5	0.2	0.3	0.2	0.6	0.3	0.7
B. Fine sand..........	5.1	0.1	0.3	0.7	1.0	1.0	0.1
C. Red clay...........	5.0	0.2	0.2	0.7	0.8	0.7	0.9
D. Coarse sand........	5.8	0.6	0.9	0.5	0.4	0.3	0.8
E. Mucky loam........	4.6	0.1	0.1	0.3	0.9	1.2	0.5
F. Neutral soil.........	7.0	0.3	0.3	0.3	0.3	0.2	
G. Alkaline soil........	7.9	0.3	0.0	0.0	0.2	0.1	

much change in hydrogen-ion concentration. The degree of buffering and the rate of change of reaction with increasing amounts of base or acid will depend very much upon soil type, as the following data will show.

Five grams each of the soils listed in table 9 were treated with 0.02 *N* $Ca(OH)_2$ equivalent to the various amounts of lime per acre of 2,000,000 pounds of soil, evaporated to dryness on the steam bath, taken up with 20 cc. of water, allowed to stand over night, and the hydrogen-ion concentration determined with a hydrogen-electrode apparatus. The acid-treated soils were managed in the same way, 0.008 *N* H_2SO_4 being used.

The tabulated data show that the rate of change of reaction with increasing increments of lime is very different for the different soils. The muck soil shows the highest buffering and the sand the least, as would be expected. The neutral and alkaline soils do not change very greatly, showing that they have little capacity for buffering against a base.

The data in table 10 show the effect of acid treatments.

As was true of buffering against bases, the organic soils show a greater capacity for buffering against acids. The sandy soil shows less buffering, and the neutral and alkaline soils have great apparent buffering power, perhaps due to the presence of excess bases.

In general, mucky or organic soils should show the highest degree of buffering, clays less, and sands the least. The protein materials of the organic soils, and the acid silicates of clayey soils are doubtless responsible for most of the buffer action of such types. Sands, containing perhaps little of either, are not usually highly buffered.

The highly buffered soils should show not only less change with the first treatments of base or acid but should continue to resist change of reaction longer when larger treatments are given. The initial reaction, of course, will be a factor to consider at this point. But it is worthy of note that the soils *A* and *C*, which are most acid to start with, show the greatest capacity for buffering against acid.

TABLE 10

Treatments and corresponding II-ion concentrations by increments corresponding to treatments

SOIL	ALONE	1000 POUNDS H_2SO_4	?000 POUNDS H_2SO_4	4000 POUNDS H_2SO_4	8000 POUNDS H_2SO	16,000 POUNDS H_2SO_4
	pH	*pH*	*pH*	*pH*	*pH*	*pH*
A. Muck	4.5	0.4	0.2	0:2	0.2	0.4
B. Fine sand	5.1	0.3	0.3	0.5	0.5	0.4
C. Red clay	5.0	0.4	0.2	0.4	0.5	0.4
D. Coarse sand	5.7	1.0	0.4	0.8	0.4	0.3
E. Mucky loam	4.5	0.3	0.3	0.3	0.3	0.3
E. Neutral	7.0	0.1	0.1	0.3	0.3	2.0
G. Alkaline	7.9	0.1	0.0	0.2	0.0	0.0

It might be supposed that since soils tend naturally to become acid the capacity for buffering against acids would be more or less exhausted. It is demonstrated that this is true to a limited extent only. While the first application of acid causes a comparatively large change in reaction, it is observed that there is a marked buffering which continues to be manifested with the highest treatments. The acid soils likewise, however, have a greater capacity for base buffering.

These facts are best brought out by means of graphs (fig. 1), which show the rate of change by the degree of curvature. Soil *A* has a curve much less steep than the other soils, soil *D* having much the most abrupt slope. The acid curves for *B* and *D* reach a final point nearly together, though starting quite widely separated. Soil *A*, which is by far the most acid, never rises to as high an acidity as soil *D*, which is by far the least acid. Soil *A* is a muck, while soil *D* is a sand and this difference in buffering capacity could be predicted, though such an extreme effect may seem extraordinary.

The above data have considerable significance in various ways. They demonstrate what practical experience has already indicated, that soils may

be quite acid when the total lime requirement is measured, and yet have a comparatively low active acidity. Ordinary soil-acidity methods measure the capacity of the soil for decomposing lime rather than its true acidity or hydrogen-ion concentration. Soils high in organic matter may be able to take up large amounts of limestone, when a great part of this acidity has been overshadowed by amphoteric substances.

Highly buffered soils also may permit vigorous bacterial activity, because the buffering effect keeps down the hydrogen-ion concentration to a point

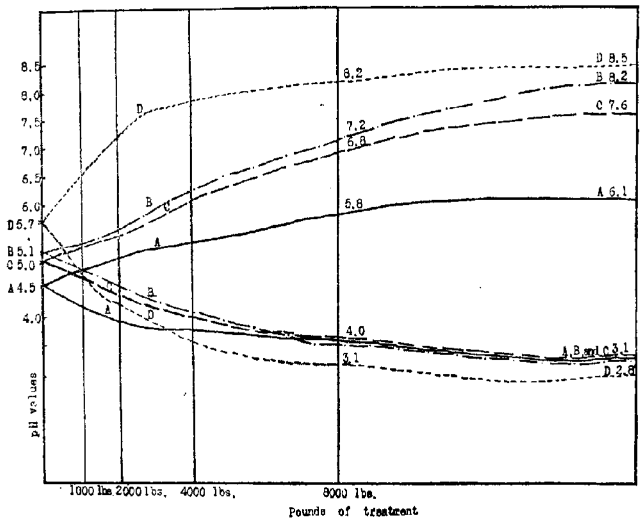

FIG. 1. TITRATION CURVES FOR SOILS A, B, C, AND D, WITH DIFFERENT AMOUNTS OF $Ca(OH)_2$ AND H_2SO_4 ADDED
$Ca(OH)_2$ Curve—upper graph
H_2SO_4 Curve—lower graph

which is not destructive of the soil organisms. A soil on the other hand which is not buffered, has a higher hydrogen-ion concentration though a smaller total lime requirement, and organisms are not very active because of the deleterious effects of the unbuffered acids.

The importance of hydrogen-ion concentration biologically may be shown by the following data taken from Fred's work (2) with legume bacteria. Only the acid limits are given, but perhaps the alkaline limit would be nearly as far above neutrality, which would mean a wide variation for some organisms and only a narrow one for others.

	acid limit pH
1. Alfalfa and sweet clover	5.0
2. Garden pea, field pea, vetch	4.8
3. Red clover and common beans	4.3
4. Soybeans and velvet beans	3.4
5. Lupines	3.2
6. Limits of growth of *Azotobacter* about	6.6 to 8.8

Many soil organisms are even more sensitive to reaction than some of the common legume organisms, and thus the true acidity of soils is doubtless the determining factor for the biological changes which are to occur. No data can be given here for the reaction permitting mold growth, but it is known that they endure high degrees of acidity and probably no soil under ordinary treatment is ever too acid for their activity.

THE NATURE OF SOIL ACIDITY

Hydrogen-ion studies

In the following brief study tumblers of soil treated in various ways were used to determine the effect of the treatment upon the hydrogen-ion concentration. In each case 100 gm. of dry soil were employed, and the moisture content kept at the optimum (50 per cent of saturation). One series was treated with ammonium sulfate at the rate of 1 ton per acre, and lime in increasing increments, 1, 3, 5, 7, 9, 12, and 20 tons per acre of 2,000,000 pounds of soil. The results are given in table 11 in pH values.

The lime requirement of the untreated soil determined by a 5-hour Tacke run was 3.2 tons per acre. It will be observed that the ammonium sulfate alone increased the acidity, as would be expected of a physiologically acid salt which has been nitrified. The increased acidity is not overcome by the 1-ton treatment of calcium carbonate, but is more than overcome by the 3-ton treatment. A neutral reaction is not secured however, until 5 tons are applied when it runs beyond neutrality. After 9 tons are applied there is only a small increase in alkalinity, and with 20 tons the pH is not quite 8.

In table 12 similar results are presented from the tests with an organic nitrogenous material, albumin, applied at the rate of 1½ tons, or approximately the equivalent in nitrogen content of the 1 ton of ammonium sulfate.

The results are very similar to those obtained with ammonium sulfate. Accidentally or otherwise, the albumin caused a slightly greater acidity when lime was not applied to the soil but in most cases it was less. In other words, the same amount of lime permitted less acidity or more alkalinity when albumin was used than when an equivalent amount of ammonium sulfate was used. This may be due to the fact that not only was nitric acid produced from ammonium sulfate but sulfuric acid also remained. When albumin was nitrified if any other acid was produced it was in a smaller quantity or more slightly ionized than the sulfuric acid from the ammonium sulfate.

TABLE 11

The hydrogen-ion concentration values for the various treatments, incubated 6 weeks

	SOIL ONLY	SOIL $(NH_4)_2SO_4$	SOIL $(NH_4)_2SO_4$	SOIL $(NH_4)_2SO_4$	SOIL $(NH_4)_2SO_4$	SOIL $(NH_4)_2SO_4$	SOIL $(NH_4)_2SO_4$	SOIL $(NH_4)_2SO_4$	SOIL $(NH_4)_2SO_4$
Lime..................	0	0	1 ton	3 tons	5 tons	7 tons	9 tons	12 tons	20 tons
pH....................	5.08	4.88	4.98	5.67	7.27	7.86	7.91	7.91	7.96

TABLE 12

Hydrogen-ion concentration values with albumin treatments

	SOIL ONLY	SOIL ALBUMIN	SOIL ALBUMIN	SOIL ALBUMIN	SOIL ALBUMIN	SOIL ALBUMIN	SOIL ALBUMIN	SOIL ALBUMIN	SOIL ALBUMIN
Lime..................	0	0	1 ton	3 tons	5 tons	7 tons	9 tons	12 tons	20 tons
pH....................	5.08	4.76	5.27	6.07	7.84	7.96	7.91	7.91	8.02

TABLE 13

Hydrogen-ion concentration values with various lime applications on soil alone

	SOIL	SOIL	SOIL	SOIL	SOIL	SOIL	SOIL	SOIL
Lime.........	0	1 ton	3 tons	5 tons	7 tons	9 tons	12 tons	20 tons
pH..........	4.70	4.91	6.55	7.69	7.68	7.90	8.05	8.26

TABLE 14

Hydrogen-ion concentration values of soils treated with acids and varying amounts of lime

	SOIL ONLY	ACIDS AND SOIL	ACIDS AND SOIL	ACIDS AND SOIL	ACIDS AND SOIL	ACIDS AND SOIL	ACIDS AND SOIL	ACIDS AND SOIL	ACIDS AND SOIL
Lime..................	0	0	1 ton	3 tons	5 tons	7 tons	9 tons	12 tons	20 tons
pH....................	4.71	3.91	4.31	6.15	7.25	7.55	7.55	7.69	7.79

TABLE 15

Hydrogen-ion concentration values on soils variously treated as shown

AMMONIUM SULPHATE					ALBUMIN			
Soil	Soil	Soil	Soil	Soil	Soil	Soil	Soil	Soil
$(NH_4)_2SO_4$ only	H_2SO_4 1 ton	H_2SO_4 3 tons	H_2SO_4 5 tons	Citric acid 10 tons	H_2SO_4 3 tons	H_2SO_4 5 tons	Citric acid 7 tons	Albumin alone
4.98	4.21	3.85	3.62	5.22	4.36	3.65	4.69	4.76

TABLE 16

Hydrogen-ion concentration values on soils treated with varying amounts of citric acid

SOIL ONLY	SOIL	SOIL	SOIL	SOIL	SOIL
	Citric acid 3 tons	Citric acid 5 tons	Citric acid 7 tons	Citric acid 9 tons	Citric acid 10 tons
pH	*pH*	*pH*	*pH*	*pH*	*pH*
4.71	5.02	5.33	5.40	5.14	5.33

In table 13 are found the results obtained where the soil alone is given the various lime treatments and the hydrogen ion determined.

Evidently there must have been some variation in the soil as this sample seems to be more acid originally. The 3-ton treatment did not produce neutrality while the 5-ton treatment produced alkalinity. Apparently about 4 tons, or a little more than the indicated Tacke requirement, is necessary to give a neutral soil. The 20-ton treatment runs above pH = 8 which is rather alkaline for a limestone treatment.

In the next series acids were added equivalent, respectively, to the nitric and sulfuric acids which would result if the ammonium sulfate were completely nitrified.

The acids increase the acidity but the 5-ton treatment of limestone gives a somewhat alkaline soil (table 14). The higher treatments do not cause as great an alkalinity as where nothing but lime is added to the soil, even when a large excess of lime is present. Another series was treated with ammonium sulfate and mineral and organic acids. Sulfuric and citric acids were used in equivalent amounts.

It is very evident that the 10 tons of citric acid in conjunction with the ammonium sulfate did not increase the true acidity of the soil (table 15). In fact, it is much less. Neither did the 7 tons used with the albumin cause any increase. But the sulfuric acid evidently caused quite a marked increase in every case, the increase being somewhat proportional to the amount applied. The 3-ton application of sulfuric acid did not have so great an effect in the presence of albumin as with ammonium sulfate, but the 5-ton treatment had nearly as great an effect.

Another series was run in which citric acid was used on the soil alone.

It is very evident again that the organic acid has not increased the acidity of the soil, and the largest application has no more effect than the smaller ones (table 16).

These results are in accord with the contention that organic acids do not accumulate in soils under conditions favorable to crop production. It is very evident that the organic acid used here has oxidized rapidly enough to remove all cause for suspicion that ordinary acid soils might owe this characteristic to citric acid produced from the decay of organic matter. The results agree also with those of Stemple, who used citric, oxalic and acetic acids. It is possible, of course, that more stable and active organic acids than citric might be produced, and that there might be conditions when such acids would contribute to the causing of an acid soil.

SOURCE OF ORGANIC AND MINERAL ACIDS

From whence arises the acidity of ordinary agricultural soils has long been a somewhat perplexing problem. It is generally believed at the present time that most of the acidity, except perhaps that in peat and muck soils, arises from some mineral source. The leaching of bases and the consequent accumu-

lation of acid silicates and alumino-silicates is doubtless responsible for a considerable portion of acidity. The practice of using certain commercial fertilizers, such as ammonium sulfate, has caused an acid condition of some soils. Thus the accumulation of sulfuric, hydrochloric, or nitric acids even in small amounts could cause a marked increase in the harmful effects of an acid soil, because such acids are highly ionized and would therefore give a high hydrogen-ion concentration. A small amount of such acids would undoubtedly do more injury than larger amounts of either acid silicates or organic acids.

It may be easily demonstrated that soils contain acids of very variable strengths, the more active ones reacting at once and the very slowly active ones only after a much longer period of contact with limestone and water.

TABLE 17

Lime requirement at intervals of 3 hours

SOIL NUMBER		3 HOURS	6 HOURS	9 HOURS	TOTAL
1	Loam (lbs.)	5000	6100	6500	6500
	Increase (lbs.)		1100	500	
	Per cent of total	77.0	17.0	6.0	100
3	Sandy loam (lbs.)	3700	5100	6200	6200
	Increase (lbs.)		1400	1100	
	Per cent of total	59.7	22.6	17.7	100
4	Sand (lbs.)	800	1100	1100	1100
	Increase (lbs.)		300	0	
	Per cent of total	72.7	27.3	0	100
5	Miami silt (lbs.)	1800	2300	2500	2500
	Increase (lbs.)		500	200	
	Per cent of total	72.0	20.0	8.0	100

This may be due to the fact that the acids are very slowly soluble, or it may be partly because of hydrolytic actions which take place slowly. The data in table 17 showing the varying degrees of activity of soil acids, are taken from a previous work.

These results show that from 60 to 80 per cent of the acidity based upon a total 9-hour run, reacted during the first 3 hours, while there yet remained 6 to 18 per cent to react during the last 3 hours of the run, except for the sand which was not very acid. Determinations have been conducted a much longer period than this and have been found to react slowly even after several days. One muck soil with a lime requirement of 15,200 pounds at the end of 3 hours gave a 25,400-pound requirement at the end of a 23-hour period. A soil of this type, however, is quite different from the ordinary soil, and doubtless the organic acids have a part to play in its reaction.

THE LOSS OF BASES BY SOILS

It is not presumed that soils become acid so long as they contain bases equivalent to the acids. But the question may arise, do acids increase in quantity or do bases diminish in quantity and thus leave a surplus acidity, and if either or both changes take place in what manner do they occur?

The bases such as sodium, potassium and calcium must be held originally in some chemical combination, undoubtedly with a silicate or alumino-silicate, to form a salt or acid salt. This gives a salt of a strong base and a weak acid and should therefore by hydrolysis give up a free base. That such is true has been demonstrated experimentally as shown by the data from Steiger's work (1) with various natural silicates (table 18).

TABLE 18

Alkalinity of natural silicates

NAME	FORMULA	COMBINED ALKALI	EQUIVALENT OF NaO IN SOLUTION
		per cent	*per cent*
Pectolite......	$Ca_2(SiO_3)_3NaH$	9.11	0.57
Muscovite....	$Al_3(SiO_4)_3KH_2$	10.00	0.32
Natrolite......	$Al_2(SiO_4)_3Na_2H_4$	15.79	0.30
Lintonite......	$Al_6(SiO_4)_6(CaNa_2)_37H_2O$	5.92	0.29
Phyogopite....	$Al(SiO_4)_3Mg_3KH_2$	9.32	0.22
Laumonite....	$Al_2SiO_4Si_3O_8Ca4H_2O$	1.00	0.18
Lepidolite.....	$KHLiAl_3(SiO_4)_3K_3Li_3(AlF_2)Al(Si_3O_8)_3$	13.00	0.18
Elaeolite......	$Al_3(SiO_4)_3Na_3$	21.17	0.16
Henlandite....	$Al_6(Si_3O_8)_6(CaNa_2)_316H_2O$	2.00	0.13
Orthoclase....	$KAlSi_3O_8$	16.00	0.11
Analcito......	$NaAl(SiO_3)_22H_2O$	14.00	0.10
Oligoclase.....	$AlNaSi_3O_8Al_2CaSi_2O_8$	9.18	0.09
Albite........	$AlNaSi_3O_8$	12.10	0.07
Wernerite.....	$Ca_4Al_6Si_6O_{25}Na_4Al_3Si_9O_{24}$	11.09	0.07
Leucite.......	$KAl(SiO_3)_2$	21.39	0.06
Stibite........	$Al_2(Si_3O_8)_2(CaNa_2).6H_2O$	1.00	0.05
Chabazite.....	$Al_2SiO_4Si_3O_8(CaNa_2).6H_2O$	7.10	0.05

These results were obtained by placing 0.5-gm. samples in 500 cc. of water and maintaining at a temperature of 70°C. for a month. It is to be expected that in the soil it might go on even more readily, since the base would be leached as liberated unless perchance it reacted with some acid or protein decomposition product to form an insoluble salt. Why all bases do not leach with about equal readiness cannot be stated, but potassium seems about the least readily leached and calcium most readily leached. When several hundred pounds of limestone may be leached out in a single year it is not strange that a soil may become rather acid and unproductive in time for that reason.

There is, therefore, nothing more logical than that with increased weathering there should come increased acidity. As long as base-rich minerals are tightly

cemented together or enclosed within the interstices of a resistant granite or other mineral, they are mechanically protected and saved from waste. But they are likewise saved from any useful function in the soil either as direct plant-food or as a neutralizing agent. Virgin soils are not only more likely to contain many minerals rich in unleached bases but they contain much organic matter in the process of decay and therefore in a condition to react with, and to prevent the leaching of base. With the exhaustion of the organic matter there is the accompanying loss of base and therefore a non-productive sour soil.

Experimental data show that practically any type of soil may become acid. But the acidity of different soil types behaves in a different way, as may be shown also experimentally. A sandy soil is likely to become acid readily because there is not sufficient organic matter to prevent leaching of such bases as may occur naturally or may be applied artificially.

There would probably be little acidity due to organic acids, because there would likely be very little organic matter in such a soil and because conditions would probably be very favorable to the oxidation of such organic acids as might possibly develop. Mineral acids such as the acid silicates, and the stronger sulfuric and hydrochloric acids from the application of certain fertilizers, would likely cause the injurious soil reaction. On a clay soil more acid alumino-silicates would be probable. Loam soils and those of yet higher organic content might contain organic acids, or at least organic compounds capable of combining with base. Such soils remain productive in spite of such acidity as may develop because necessary bases for plant growth have been prevented from leaching and because the organic matter itself is an important source of the essential plant-food, nitrogen. In the growth of legumes, however, it is perhaps not a question of nitrogen content, but more likely a question of reaction and a supply of mineral plant-food, including not only the bases potassium and calcium, but also phosphoric acid.

GENERAL DISCUSSION

There are many factors which influence directly or indirectly the reaction of soils. It is not alone a question of the production of acids but a question of the capacity of the soil to resist changes in reaction caused by the acids produced.

Buffering may be effected by both mineral and organic compounds. Silicates of bases would be capable of neutralizing strong acids, which is in fact a buffering effect. Some of the alumino-silicates no doubt react with either acid or base and therefore function doubly, saving base and reducing the true acidity. The amino acids and many more complex products of protein degradation react in the same manner. The ionization constants for the amino acids as either acids or bases are very low but of about equal strength, making them ideal buffers. This explains why organic soils and clayey soils should show greater power to resist changes in reaction.

Grain size is of course an important factor in determining its reaction, especially with mineral soils. The smaller the grain size the more difficult it is to prevent water-logging, and therefore the more difficult to maintain conditions favorable to the oxidation of organic acids or other harmful products. Though coarse-grained soils readily become acid it is perhaps usually with a somewhat different type of acidity. Rahn (3) has already demonstrated the close relationship between grain size, moisture content, and bacterial activity. This relationship has its influence also upon reaction changes.

SUMMARY

1. Highly organic soils and clays exhibit a high degree of buffering, while coarse sands show little of this capacity.

2. Sulfuric acid, or physiologically acid salts such as ammonium sulfate, cause a change toward increased hydrogen-ion concentration in soils. Citric acid did not increase the true acidity.

3. Ammonium sulfate caused a greater increase in acidity than did its nitrogen equivalent of albumin.

4. When nitric and sulfuric acids were added to the soils in amounts equivalent to the acidity which might be produced from the complete nitrification of ammonium sulfate, a greater increase was produced in the hydrogen-ion concentration of the soil than where the ammonium sulfate was used.

5. A large excess of pure lime carbonate (20 tons) brought the pH value to only a little more than 8.0, which seems to be about the limit of alkalinity produced by limestone.

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THE ROLE OF OSMOTIC PRESSURE IN THE TOXICITY OF SOLUBLE SALTS

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The following brief résumé of the literature shows that many investigators consider the toxicity of soluble salts toward bacteria and higher plants to be due mainly to an osmotic effect. Stolgane (23) found that plants could resist only a comparatively low osmotic pressure, which, when not exceeding two atmospheres, stimulates growth. However, all the vegetative processes including growth may be checked entirely if the pressure becomes much higher than two atmospheres.

Ewart (7) found that heavy dressings of salt retarded the germination of seeds and in some cases caused them to rot in the soil. The injurious action of the salt he attributed mainly to osmotic influences.

Maliuschitskii (19) grew cereals in sand cultures with a normal nutrient solution to which were added various compounds of calcium, magnesium, sodium and potassium, comparison being made between each individual salt. In one set the solutions were isotonic, whereas in the other series the strength of the solutions was raised to the same degree of isoconcentration. He found that when isotonic solutions were used the plants resembled one another in external appearance in the amount of dry matter and in anatomical characters as well as in the percentage of nitrogen they contained. When, on the other hand, plants were grown in isoconcentrated solutions, especially in the case of such salts as magnesium sulfate and sodium chloride, the plants differed not only in their nitrogen content but in other respects. Similar results were obtained later with sugar-beets, oats and millet.

Beauveria (4) observed that the structure of *aspergillus*, *phosealus*, *pesun*, *lupinus*, *zea*, and *triticum* were modified by changes in osmotic pressure.

Hill (15) studies various salt-marsh plants to find the effect of differences in the salinity of the soil upon the plants and how the plant accomodates itself to the fluctuating concentration of the soil water.

The results indicated that the root-hairs of *salicorna*, growing in places where the soil water is strongly saline, can adapt their internal osmotic pressure to the osmotic strength of the soil solution. On transferring isolated seedlings from relatively strong saline solutions to fresh water, the tips of the root-hairs were found to swell and assume abnormal shapes. Branching was also occasionally seen. The cellulose walls of the tip of the root-hair under

some conditions became thickened. This he believed to be of value in giving the end a greater strength in order to protect against a sudden increase in the internal pressure.

According to Tulaikov (24) soluble non-nutritious salts of the soil have a noteworthy influence on vegetation from the first stages up to harvest. At germination the non-nutrients appear to exercise an essential physical action in modifying osmotic pressure, thus regulating the absorption of water by the germinating seeds. Isotonic solutions of different salts have an almost equal action. The toxic effect of the different salts on seedlings of various plants is in general due to plasmolysis. In some cases there is a chemical action since the tissues of the root system are destroyed or its superficial cells modified.

Barnes and Ali (3) consider the changed osmotic pressure to be the main cause of injury to plants and even bacteria caused by alkali salts, as seen from the following:

> From our own observations we conclude that the salts' presence in alkali soils does not exert any toxic effect on the plant. The effects produced are purely physical. . . . . The danger point is reached when the osmotic pressure of the saline solution becomes equal to that of the cell sap. This is irrespective of the nature of the salt, provided it possesses no toxic properties; such salts as the sulfates and chlorides of sodium or calcium exhibit no toxicity to plant protoplasm. Even carbonate of soda cannot be said to be toxic in the present state of our knowledge, though we know that it shows evidence of caustic action on the stems of plants growing in soils affected by the salt. Whether the caustic effect is produced on dead or living tissue is not known. But as soon as the osmotic pressure of the fluid entering the root tips from the soil becomes greater than that of the cell sap, the protoplasm of the cell shrinks away from the containing walls and the plant loses its turgidity and becomes flaccid. It shows in fact all the appearance of withering. and if the concentration of the external fluid is not immediately reduced by dilution the plant dies. Dilution of the saline solution is in itself a remedy at this stage, and this is one reason which leads us to believe that the salts possess no toxic properties and that their effect is a purely physical phenomenon.

Hansteen (12) found that magnesium, sodium, and potassium salts in the cultural fluid caused the roots of wheat to shrivel and die, whereas the presence of calcium salts increased the root growth. The magnesium ions were the most toxic, but he found that neither temperature, osmotic pressure, nor the anions present influenced the toxicity.

Harris (13) found there was little relationship between the molecular weight of a compound and its toxicity to wheat seedlings and he therefore concludes that the osmotic pressure is not the controlling factor in the toxicity of the common soil alkalies.

Klebs (16) and other workers found that many green algae were capable of existing in two or more forms, but the stimuli inducing the change were unknown. Livingston (17), however, showed that it was the osmotic pressure of the solution in which the algae grew. He demonstrated that the response of *Stigeoclonium tenue*, both in form and in reproductivity, which

accompanies a change in concentration of Knop's solution in which it is growing, is due to changes in the osmotic pressure of the medium and is in no way a function of its chemical composition. Further work (18) demonstrated the fact that solutions of non-electrolytes produce the same results as those of electrolytes, for in them, also osmotic, is the controlling factor in determining the form of the plant. This is effective through changes in the water content of the cells.

Livingston (18) found that a high osmotic pressure influences the plant in four ways: (a) it decreases vegetative activity; (b) it inhibits the production of zoospores; (c) it causes cylindrical cells to become spherical; and (d) it frees the algae from certain limitations as to the orientation of the planes of cell division. Conversely, a low pressure reverses these effects. It was also ascertained that there were certain quantitative differences between the concentrations required to inhibit zoospores in the palmella and in the filamentous stage, those required for the former being the greater. Between the behavior in this respect of electrolytes and of non-electrolytes, such as sucrose, quantitative differences were found.

Atkins (2), however, considers it improbable that all these phenomena are due merely to variations in the amount of water in the vacuoles. He considers it associated with the alterations in the state of inhibition of the protoplasmic colloids, also with changes in the rate of oxidations which normally occur in the cell, owing to increases or decreases in the area of external surface, and consequent disturbance of the usual rate of intake of atmospheric oxygen. Artari (1) is also of the opinion that various salts have other effects upon algae than the osmotic.

Many bacteria lose their motility when brought into solutions of a high osmotic pressure, and Wladimeroff (25) has employed this arrest of motility to measure osmotic pressure.

Barnes and Ali (3) consider that bacteria are subject to the same physical laws of molecular pressure in solution as the higher plants or the dead membrane of the physicist's instrument, and hence are destroyed by osmotic changes.

THEORIES AS TO TOXICITY

Overton (22) considered inorganic salts incapable of penetrating living cells, but Osterhout's experiments (21) on spirogyra clearly demonstrated that they do. He further found that sodium chloride and other salts of monovalent metals increase the permeability of protoplasm, whereas calcium chloride and the salts of other divalent metals have just the opposite effect. Where cells are placed in a mixture of the mono- and divalent salts there is a mutual hindrance of each salt upon the entrance of the other into the cell; hence, the toxic action of each salt upon the protoplasm is diminished by the presence of the other, because their rate of penetration into the protoplasm is greatly reduced.

Some ions on entering the cell may produce a colloidal precipitate in the root cell and this either retards the growth or causes the death of the plant, depending upon the nature and concentration of the medium.

De Lavison (6) found that for weak solutions cations of alkalis and alkaline earths combined with non-toxic anions penetrated the protoplasm with difficulty. Certain salts of aluminum and yitrium and a large majority of the salts of the heavy metals do not penetrate the living protoplasm. He held that the permeability of the protoplasm to weak solutions is an absolutely different phenomenon from its permeability by strong solutions. The protoplasm under the influence of strong solutions becomes completely permeable, without, however, being killed by those salts which in weak solutions are unable to penetrate it. He claims to have established two facts as to toxicity of salts.

(a) The toxic action of a molecule is approximately a property of acid and basic radicals for a large number of salts, while this is due to properties acquired by the molecule by reason of its non-saturation by the acids and bases.

(b) The toxic salts are those which do not penetrate at all or only with difficulty the living protoplasm when they are employed in weak solutions, whereas solutions of non-toxic salts, on the contrary, easily penetrate the protoplasm. The protoplasm seems to be an unstable substance as regards a large number of salts.

Undoubtedly the action produced varies with the specific salt. Fluri (8) considers that aluminum salts act upon the diastase, thus reducing the production of starch. There may even be a different effect exerted by the negative or positive ion of the same salt. Nabokich (20) found that the anions of most compounds produce a strong acceleration, whereas the cation hinders or limits growth.

It is quite evident from the preceding brief review of the physical, chemical and colloidal theories which have been offered for the toxicity of salts that many workers lean to the physical with the dominant idea that in many cases at least the predominating factor is an osmotic phenomenon, and it became evident to the senior author of this paper early in his study of the influence of soluble salts upon the bacterial activities of the soil, that while the osmotic pressure, plays an important part in the toxicity of most salts to bacteria, yet the physiological influence exerted upon the protoplasm of the cell is not to be ignored.

METHOD OF INVESTIGATION

The toxicity of the chlorides, carbonates, nitrates and sulfates of sodium, potassium, calcium, magnesium, manganese and iron to ammonifying and nitrifying bacteria was determined by the regular beaker method (9, 11). From the data thus obtained we were able to compare the compounds as to toxicity from three viewpoints: (a) the lowest concentration of the salt at which a toxic effect is noted toward the ammonifying and nitrifying organisms

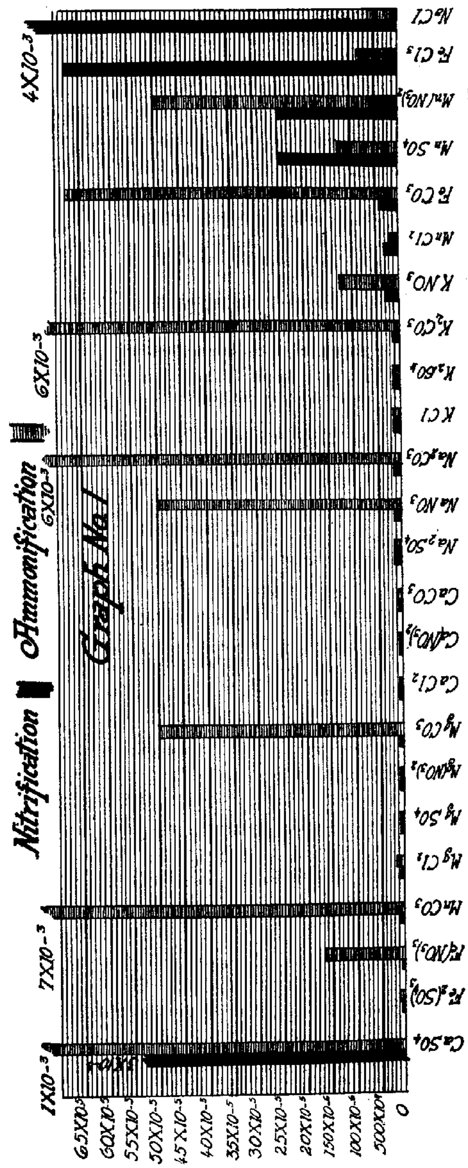

FIG. 1. GRAPHS SHOWING THE MOLECULAR CONCENTRATIONS AT WHICH THE VARIOUS SALTS ARE TOXIC TO AMMONIFYING AND TO NITRIFYING ORGANISMS

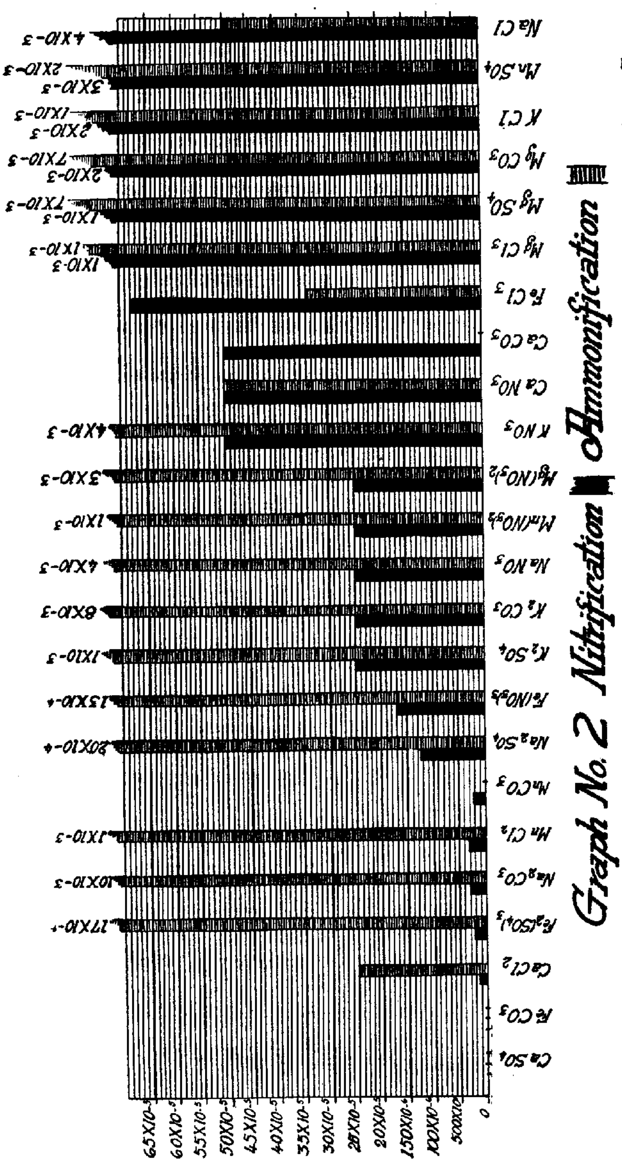

FIG. 2. GRAPHS SHOWING THE MOLECULAR CONCENTRATIONS AT WHICH AMMONIFICATION AND NITRIFICATION ARE REDUCED TO ABOUT THREE-FOURTHS NORMAL

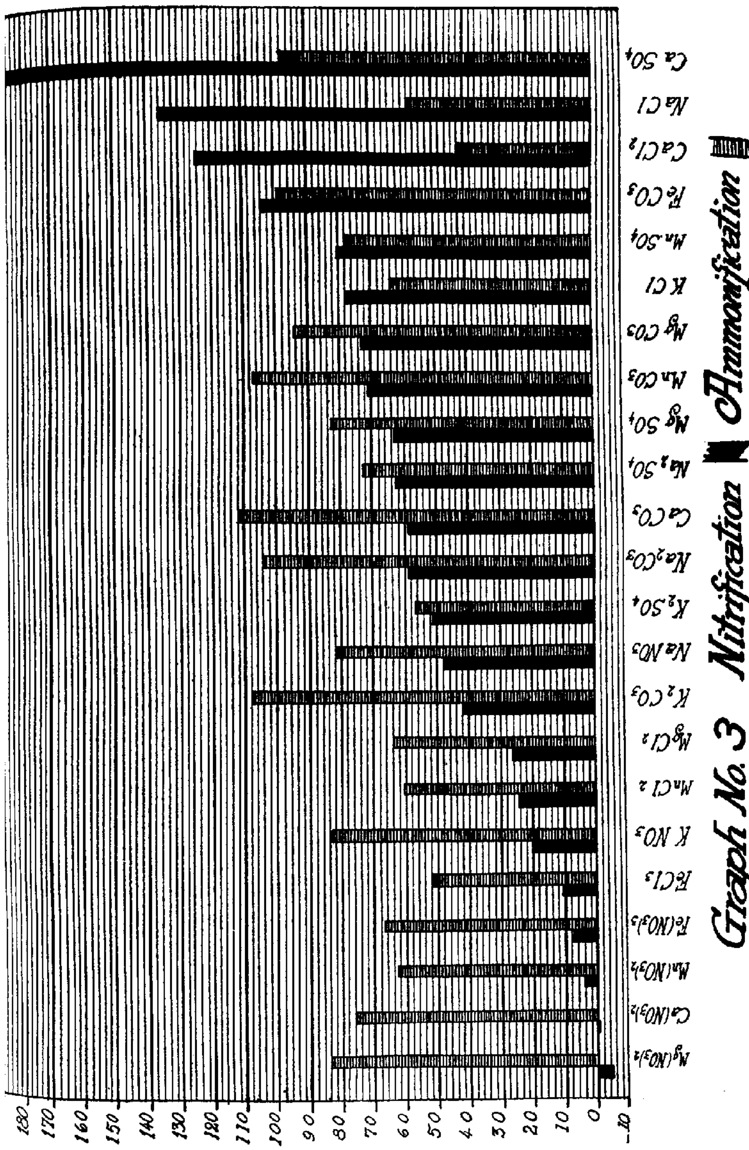

FIG. 3. GRAPHS SHOWING THE PERCENTAGES OF AMMONIA AND NITRIC NITROGEN PRODUCED IN 100 GM. OF SOIL TO WHICH HAD BEEN ADDED 2×10^{-3} M OF THE VARIOUS SALTS, THE UNTREATED SOIL BEING COUNTED AS PRODUCING 100 PER CENT

(fig. 1); (b) the molecular concentration at which ammonification and nitrification were reduced to three-fourths normal (fig. 2); and (c) the percentages of nitric nitrogen and ammonia produced in the presence of the largest quantity of the various salts, which is 2×10^{-3} *M* of the salt in 100 gm. of soil (fig. 3)

Not one of the compounds tested was toxic at the lowest concentration—78×10^{-7} *M*. All became toxic at some of the concentrations tested. In eleven out of the twenty cases the point of toxicity for the ammonifiers and nitrifiers was the same, whereas in the remaining cases the quantity required to become toxic to the ammonifiers was much greater than it was for the nitrifiers. In only three instances were the salts more toxic to ammonifiers than to nitrifiers.

A relationship between the toxicity of the compound and its power to precipitate colloids was not found. It appears, therefore, that while the precipitation of the colloidal cellular material often causes the death of the organisms, it is not necessarily the determining factor in the toxic action of these salts.

As can be seen from figure 3, it is not necessarily these compounds which become toxic at the lowest concentration which have the most far-reaching effect upon the bacterial activities of the soil. This condition holds for both the ammonifying and nitrifying organisms. It requires in almost every case more of the specific salts to reduce ammonification to three-fourths normal than is required to produce the same effect upon the nitrifiers.

The osmotic pressure of the soil is not necessarily directly proportional to the quantity of salt added to the soil, as some salts may ionize to a greater extent than others. Moreover, the addition of a salt to a soil changes materially its soluble constituents (10), and hence may either increase or decrease its osmotic pressure. Therefore, measurements were made of the osmotic pressure of the soil under as nearly as possible the same conditions as maintained when bacterial activity was determined. This was done by means of the cryoscopic and conductivity methods.

Cryoscopic method

One-hundred-gram portions of the soil were mixed in tumblers with the quantity of salts indicated in figures 1, 2, and 3. To these were added 2 gm. of dried blood and their moisture content brought to 18 per cent. These were allowed to stand in covered tumblers for 12 hours and then transferred to a tin tube (*2*, fig. 4), in which the freezing point was determined.

The determinations were made by the method as outlined by Bouyoucos (5) except that we used ether in place of ice for the freezing of the soil. The ether was evaporated from a glass container insulated with kieselguhr (fig. 4) by drawing air through *3* by means of a tube, *4*, connected with an air pump.

Ether was economized by conducing cold air through tube 3 from the outside of the building. The tubes containing the soil were placed in an ice-salt

mixture before placing in the ether, thus removing the greater excess of heat before attempts were made to freeze. By following this procedure it was found possible to freeze a sample of soil with half an ounce of ether. This method has the advantages over the regular ice-salt mixture in being easier

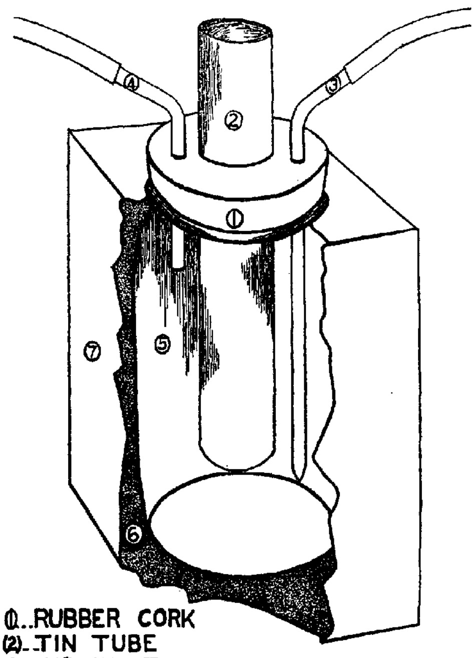

FIG. 4. DRAWING OF CONTAINER USED FOR FREEZING SOIL

to manipulate and quicker; furthermore it does not cloud the thermometer, thus permitting a more accurate reading.

In commencing a determination it was found advisable to have the temperature of the ether not lower than $-2°C.$; otherwise, the soil will not supercool, and hence the freezing point cannot be accurately read.

Conductivity method

The soil was prepared as in the cryoscopic method and the conductivity determined as outlined by Hibbard (14). The apparatus used was a transformer, an alternating current galvanometer, a bridge, a Curtis coil, and resistance box. The scheme of connection is given in figure 5.

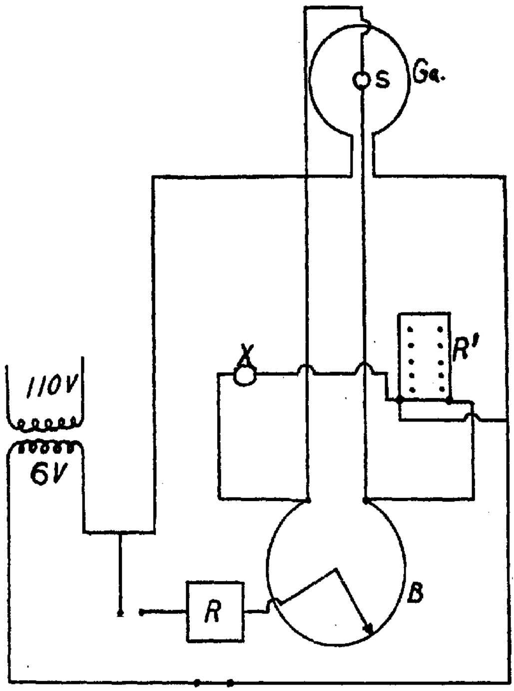

Fig. 5. Scheme Showing Method of Connecting Apparatus for Determining Conductivity

B—Wheatstone bridge; *R*—resistance coil for cutting down current; *R'*—known resistant coil; *Ga*—alternating current galvanometer; *S*—movable coil and galvanometer 110 v. to 6 V.

The source of current was the alternating current of the electric light. The terminals which were placed in the soil were made from two spatulas the blades of which were cut to 3 inches and tipped with platinum foil, the remaining part of the blade being covered with a good insulator. The handles were insulated and solidly bound together. These were plunged into the soil of the tumblers. The whole was kept at a constant temperature within 0.01°C.

by means of a thermostat, the outline of which is shown in figure 6. The temperature was held constant for 30 minutes before determining the conductivity. All determinations were made in triplicate, and the results as reported are in every case the averages of six or more closely agreeing determinations.

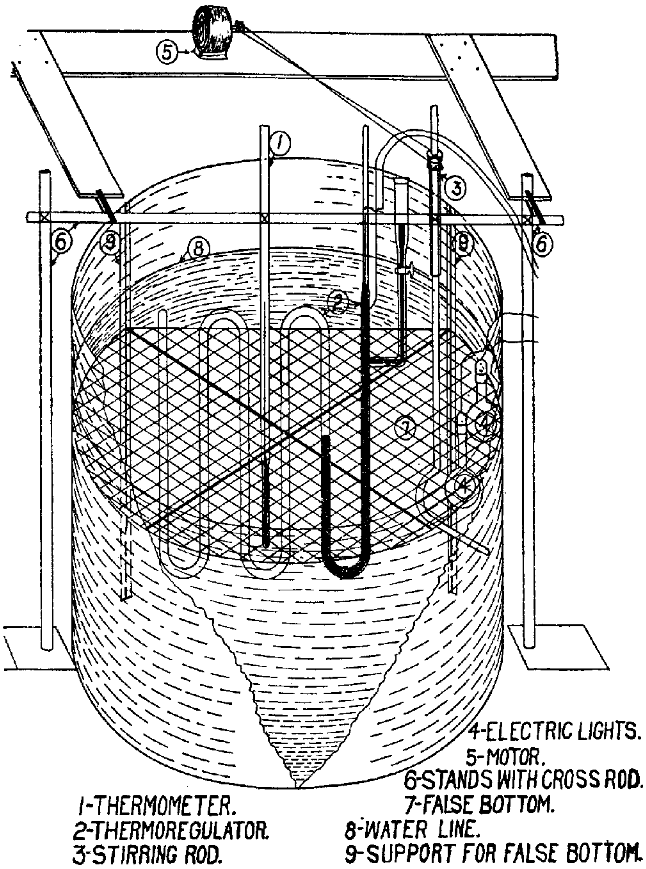

Fig. 6. Drawing Showing Construction of Constant-Temperature Bath

AMMONIFICATION

Figure 7 gives the relationship between the osmotic pressure as determined by the freezing-point method and the conductivity method of a soil to which sufficient salt had been added to become toxic to the ammonifying organisms.

On the ordinate is given the per cent of ammonia formed, the untreated soil being taken as 100, whereas on the abscissa is given the osmotic pressure in atmospheres. With the exception of iron chloride the osmotic pressure is higher and slightly more uniform as determined by the conductivity method than by the freezing-point method. Calcium nitrate, manganese nitrate, and sodium carbonate with both methods yield abnormal results, probably due to the action of these salts upon the organic and inorganic constituents of the soil. Throughout the work it would appear that the most reliable and coordinate results are obtained with the conductivity method.

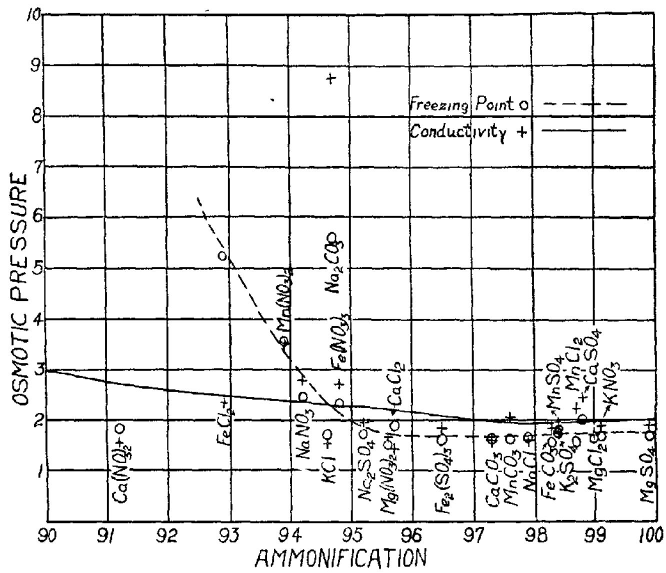

FIG. 7. GRAPHS SHOWING OSMOTIC PRESSURE AND MILLIGRAMS OF AMMONIA PRODUCED IN SOIL TO WHICH THE VARIOUS SALTS WERE ADDED IN QUANTITIES SUFFICIENT TO BECOME TOXIC TO AMMONIFYING ORGANISMS

The other compounds show a marked relationship between the osmotic pressure of the soil solution and the toxicity of the salt toward ammonifying organisms. With the exception of those salts noted, all become toxic when they produce within the soil an osmotic pressure of from $1\frac{1}{2}$ to $2\frac{1}{2}$ atmospheres, thus indicating that the toxicity of these salts is due mainly to increased osmotic effects.

However, as the osmotic pressure of the soil is increased by the addition of the several salts the relationship observed above is not so uniform, as may be seen from figure 8. In this figure is given the percentages of ammonia pro-

duced and the osmotic pressure of soil to which sufficient salt had been added to reduce ammonification to three-fourths normal. Bacteria produce ammonia in a soil the osmotic pressure of which is from 8 to 15 atmospheres, when the osmotic pressure is due to sodium carbonate and the nitrates of magnesium, sodium and iron. When the osmotic pressure is due to the nitrate and chloride of calcium the sulfate and chloride of iron, sodium chloride, magnesium carbonate, potassium chloride, and manganese sulfate, the ammonia production is reduced to three-fourths normal with an osmotic pressure below four

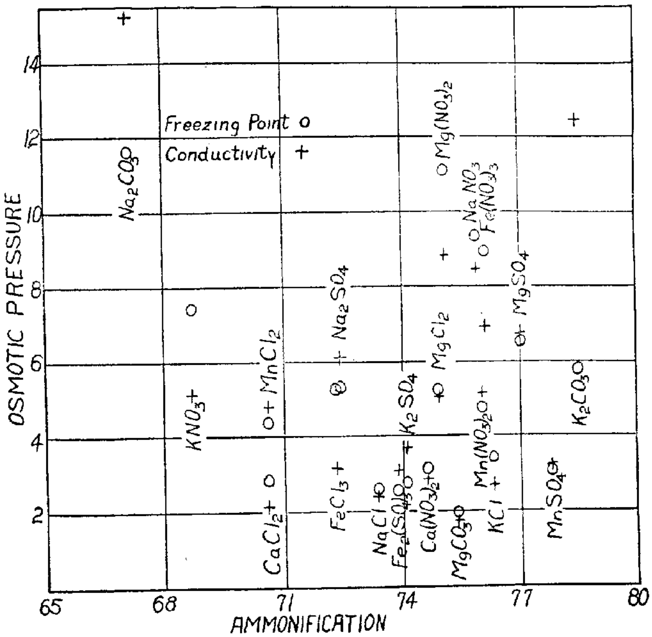

FIG. 8. GRAPHS SHOWING OSMOTIC PRESSURE AND MILLIGRAMS OF AMMONIA PRODUCED IN SOIL TO WHICH THE VARIOUS SALTS WERE ADDED IN QUANTITIES SUFFICIENT TO REDUCE AMMONIFICATION TO ABOUT THREE-FOURTHS NORMAL

atmospheres. This indicates that whereas the beginning of toxicity of the several salts toward ammonifying organisms is due largely to osmotic pressure, as the concentration of the salt increases other factors enter, but even in very high concentrations there is a striking relationship between osmotic pressure and ammonia produced in the soil, as may be seen in figure 9. It is surprising to find that ammonifying organisms can function in soils having osmotic pressures as high as 45 atmospheres. Under this condition, however, the quantity of ammonia produced is very small.

The results taken as a whole indicate that the toxicity of the several salts examined is due to two factors: (a) the increased osmotic pressure produced by the salt within the soil and (b) a probable physiological action of the substance upon the living protoplasm of the cell changing its chemical and physical properties so that it can no longer function normally. The former factor is probably the more far-reaching. This is the reverse of the early conclusion (9, 10, 11), for the osmotic pressure is not always directly proportional to the salt added but depends upon the kind and degree of association. Furthermore, many salts undergo double decomposition within the soil, thus markedly changing what would otherwise be the osmotic pressure.

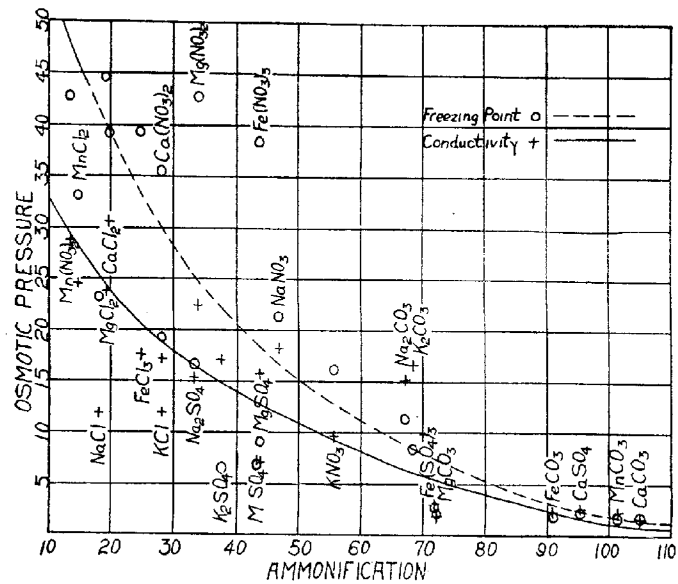

FIG. 9. GRAPHS SHOWING OSMOTIC PRESSURE AND AMMONIA PRODUCED IN SOIL TO WHICH 10×10^{-3} *M*. OF THE VARIOUS SALTS WERE ADDED TO 100 GM. OF SOIL

NITRIFICATION

The osmotic pressure was determined by both cryoscopic and conductivity methods on soil to which sufficient salts had been added, first to become just toxic to nitrifying organisms, second to reduce nitrification to three-fourths normal, and third to produce the highest concentration of the salt which was studied in the nitrification test of toxicity—2×10^{-3} *M* in 100 gm. of soil.

Figure 10 gives the results obtained when sufficient salt was added to become toxic. On the ordinate is given the per cent of nitric nitrogen formed, whereas

on the abscissa is given the osmotic pressure as determined by the two methods. It is quite evident that there is a large difference in the extent to which the various salts reduce nitrification. Some of them yield 100 per cent or above at the one concentration, and at the next concentration tested, a great decrease in nitrification is observed. Yet it is quite evident in the case of most of the salts that there is a relationship between the osmotic pressure of the soil and the toxicity of the several salts—sodium chloride, iron chloride, and manganese nitrate being clearly exceptions. The osmotic pressure at which nearly all salts become toxic to nitrifying organisms is between 1 and 2

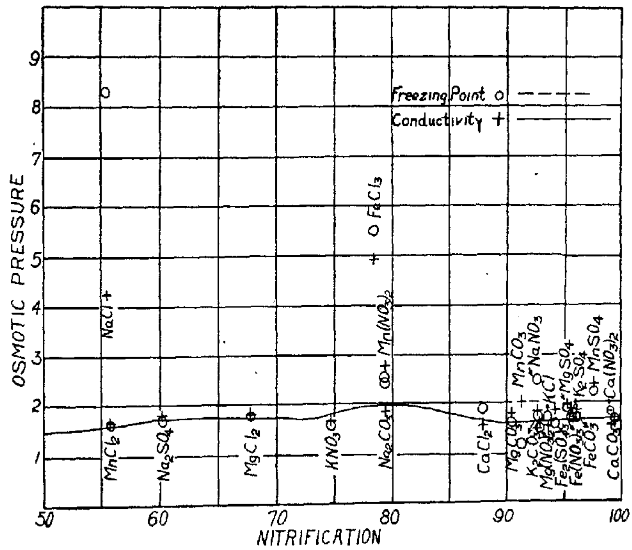

Fig. 10. Graphs Showing Osmotic Pressure and Milligrams of Nitric Nitrogen Produced in Soil to Which the Various Salts were Added in Quantities Sufficient to Become Toxic to Nitrifying Bacteria

atmospheres. In the case of the ammonifying organism it ranges between 1 and 3 atmospheres, usually averaging slightly higher for ammonifying than for nitrifying organisms. As the concentration of the salts increases the difference in sensitiveness of the two groups of organisms becomes more divergent. This may be seen from figure 11. As an average, all of the salts reduce nitrification to about three-fourths normal when the osmotic pressure of the soil ranges between 2 and 3 atmospheres, whereas for ammonification it is considerably higher than this. It is quite evident here also that sodium chloride and iron chloride do not as closely conform in toxicity and osmotic

pressure as do the other salts. The results as a whole, however, point to the conclusion that where the toxicity of the tested salts is due primarily to osmotic effect, yet physiological influences undoubtedly play a part. The latter part of this conclusion is well borne out by the results given in figure 12. Here we find calcium sulfate producing an osmotic pressure of two atmospheres, and still the nitrifying organism produces twice as much nitric nitrogen as in untreated soil. Soil to which sufficient sodium chloride had been

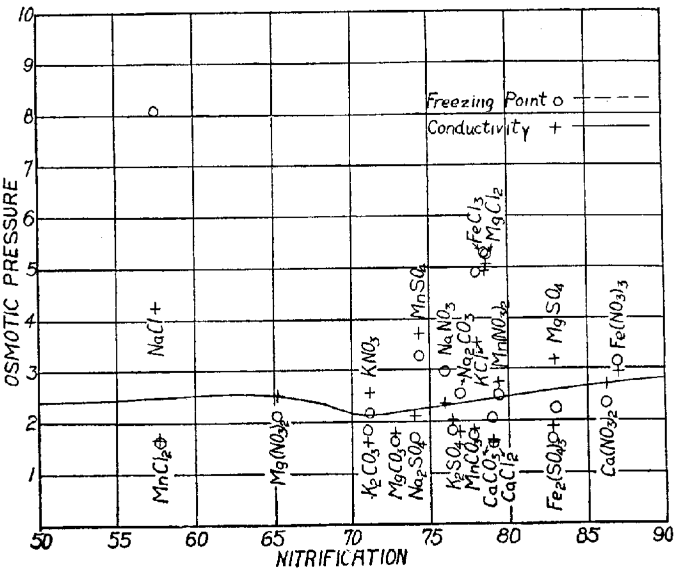

FIG. 11. GRAPHS SHOWING OSMOTIC PRESSURE AND MILLIGRAMS OF NITRIC NITROGEN PRODUCED IN SOIL TO WHICH THE VARIOUS SALTS WERE ADDED IN QUANTITIES SUFFICIENT TO REDUCE NITRIFICATION TO ABOUT THREE-FOURTHS NORMAL

added to produce a like osmotic pressure showed 140 per cent normal nitric nitrogen. Yet a similar osmotic pressure produced by potassium carbonate or potassium nitrate nearly stops nitrification.

It is quite evident that the nitrifying organisms are much more sensitive to osmotic changes than are ammonifying organisms. No nitrates were produced in soils having an osmotic pressure of over 8 atmospheres, whereas ammonification took place to a small degree in soil the osmotic pressure of which was 35 atmospheres.

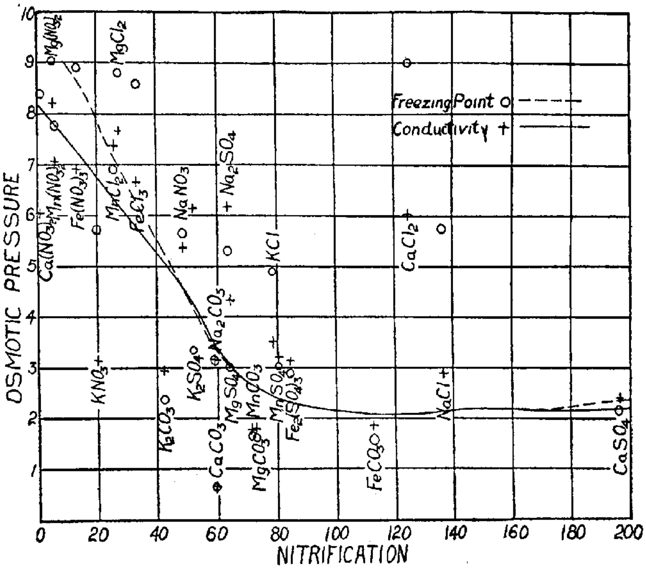

FIG. 12. GRAPHS SHOWING OSMOTIC PRESSURE AND NITRIC NITROGEN PRODUCED IN SOIL TO WHICH 2×10^{-3} *M*, OF THE VARIOUS SALTS WERE ADDED TO 100 GM. OF SOIL

SUMMARY

The osmotic pressure was determined by the cryoscopic and electrical conductivity methods on soil to which the chlorides, sulfates, carbonates and nitrates of potassium, sodium, caldium, magnesium, iron and manganese had been added in quantities such that (a) the salts became toxic to the ammonifying organism, (b) the quantity of ammonia produced in unit time was reduced to three-fourths normal, (c) the concentration of the salt in the soil was 10×10^{-3} *M* per 100 gm. of soil, (d) the salt became toxic to the nitrifying organism, (e) the nitric nitrogen produced in unit time was reduced to three-fourths normal, and (f) there would be 2×10^{-3} *M* of the salt in each 100 gm. of the soil.

With the exception of manganese nitrate, iron nitrate and sodium carbonate there is a close correlation between toxicity and osmotic pressure. All the salts tested, except these three, became toxic when the osmotic pressure was less than 3 atmospheres.

As the concentration of the salt added to the soil increases it is evident that the retarding effect upon the ammonifying organism is not due entirely to the osmotic pressure. There is probably a physiological action of the substance upon the living protoplasm, changing its chemical and physical properties so that it cannot function normally.

All salts tested reduced ammonification to less than one-half normal when the osmotic pressure of the soil reached 15 atmospheres. Yet there were appreciable quantities of ammonia produced in the presence of some salts when the osmotic pressure reached 20 atmospheres.

With the exception of sodium chloride, manganese nitrate, and iron chloride, all the salts tested became toxic to nitrifying organisms when the osmotic pressure ranged between 1 and 2 atmospheres.

The nitrifying organisms behave in a manner similar to the ammonifying organisms, except that they are retarded at much lower osmotic pressures.

All the salts reduced nitrification to less than 50 per cent when the osmotic pressure reached 6 atmospheres.

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THE EFFECT OF FERTILIZER TREATMENTS ON SAVANNAH CRANBERRY LAND[1]

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The New Jersey Agricultural Experiment Station has been carrying on fertilizer experiments on cranberry land for 8 years. Before this period the growers were using some commercial fertilizer in rather conservative amounts, but with little idea of the proper proportion of the various ingredients. The station administration felt that it should have definite data to meet the requests for information from the growers. Following an offer of coöperation by the American Cranberry Growers' Association in the spring of 1913 the work was at once begun and has continued to the present time.

THE CRANBERRY BOG

The cranberry plant is so unusual that a few words relative to its culture and management may be necessary for a complete understanding of the fertilizer studies. The cranberry is one of the heath plants native to acid swamps of northern North America. Under cultivation it is grown rather widely throughout the southern part of New Jersey as well as in eastern Massachusetts and in Wisconsin. Before setting out the plants, the ground is cleared of all its natural growth, the stumps and roots are removed and the ground at least roughly leveled. Cranberries are propagated by hard-wood cuttings, set out in squares from 10 to 24 inches apart. The cuttings send out runners which entirely cover the ground and which send up "uprights" bearing fruit buds. The first harvest is on vines 4 years old and after that crops may be expected annually for 50 years. The character of the vines precludes any cultivation in the ordinary sense of the word.

Cranberry bogs are submerged in winter from December to the first week in May to prevent winter injury, to control certain insect pests and to secure an even start of the vines in spring. They are "reflowed" usually about the first of June to control other insect pests. Flooding for frost protection is practiced as often as the weather demands. After harvest the bogs are put under water for a week for cleaning up various pests.

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The cranberry is in full bloom the first week of July and the harvest starts early in September. Fertilizer applications should be broadcasted directly after the June reflow if the treatment is to be fully effective the year of its application.

CRANBERRY SOILS

Cranberry soils are of three different types, Savannah, mud and iron-ore bottom. Geologically, all of are alluvial origin of comparatively recent formation. All are underlaid with a hard-pan very impervious to water. The Savannah is made up of a coarse sand together with enough organic matter to give it a black color. The native growth on Savannah consists chiefly of leather leaf or cassandra (*Chamaedaphore calyculata*). The mud is the peaty substance deposited in the bottom of cedar swamps, and may be from a few inches to 20 feet thick. Iron-ore bottom has the same general appearance as mud, but just below the surface a substance rich in iron, known locally as bog iron ore, is found. The Savannah is the only soil discussed in this paper.

METHODS USED

Absolutely controlled conditions such as are used in pot experiments with upland crops are impracticable with cranberry soils, because of the nature of the plant and its management. A uniform covering of cranberry plants is necessary to determine the ability of the soil to produce fruit, and such a covering would require 6 to 8 years. The regular flooding for insect pests and the careful irrigation are features hard to reproduce in the greenhouse. The relation of the long winter flooding to residual plant-food in the soil would be a difficult problem.

Field experiments where treatments were made on plants already in bearing and allowing for numerous untreated plots seemed to meet the more important needs of the situation. However, constant inspection of such an experiment is necessary in order to evaluate outside factors, such as insect injury and fungus disease.

In reporting results, the treated plot is compared with the average of the two untreated plots between which it is located. The following is given as an example:

PLOT	TREATMENT	YIELD	GAIN
		lbs.	*per cent*
51	Nothing	50	
52	300 pounds nitrate of soda	60	$33\frac{1}{3}$
53	Nothing	40	

$$60 - \frac{40+50}{2} = 60 - 45 = 15 \qquad \frac{15}{45} = 0.33\tfrac{1}{3}\text{, or } 33\tfrac{1}{3} \text{ per cent}$$

The treatment and the yield are given invariably in pounds per acre.

MATERIAL	ANALYSIS OF SUBSTANCES USED
Nitrate of soda	15½ per cent nitrogen
Ammonium sulfate	20½ per cent nitrogen
Dried blood	10 per cent nitrogen
Cottonseed meal	5 per cent nitrogen
Acid phosphate	16½ per cent available "phosphoric acid"
Basic slag	19 per cent phosphoric acid
Rock phosphate	30 per cent total "phosphoric acid"
Steamed bone	1½ per cent ammonia; 60 per cent phosphoric acid
Bone meal	2 per cent ammonia; 30 per cent phosphoric acid
Muriate of potash	50 per cent "actual potash"
Sulfate of potash	48 per cent "actual potash"
Kainit	12 per cent "actual potash"
Calcium cyanamide	19 per cent nitrogen
Barium phosphate	25 per cent total "phosphoric acid"

EFFECT OF DIFFERENT KINDS OF PLANT FOOD

The first work in determining a cranberry fertilizer was to find the kinds of plant-food to which the cranberry plant responds. Plant-food from various common sources was applied to typical Savannah land and the results recorded. The first treatments were made in 1913. Nitrogen at the rate of 40 pounds to the acre, phosphoric acid 80 pounds, and potash 100 pounds, both alone and in combination with each other, were applied annually. After 3 years the vegetative growth became so great that it was concluded that the applications were too large, and further treatments were omitted until 1918. In this year half the original amounts of nitrogen and phosphoric acid were used, no potash was added in 1919, and 1920 no treatments were made.

The results set forth in table 1 represent increases over check plots for each year.

Insect enemies have a peculiar way of attacking a partly fertilized bog. Not only do they attack the well fed vines simply because of the more numerous tips, but also on account of the more trash on the floor of the bog. This latter condition especially meets the requirements of the blossom worm. In 1914, there was a general attack from this pest, causing a falling off in the yield of all the fertilizer plots, and in 1919 the attack was so concentrated on the treated plots that practically the entire crop was taken. For this reason, no crop record was made in 1919. Another insect, the cranberry girdler, finds a home on the treated plots and soon manages to reduce the crop and sometimes entirely kills the vines on the plots attacked. It was this insect that caused the loss on plots 25, 27 and 29. Of course, if these insects attack the check plots as well as the treated plots, there would be no need of making the special note of them, but their attack is almost entirely localized on the treated plots.

TABLE 1

Summary of effects of various fertilizer mixtures on the yield of cranberries

PLOT	TREATMENT PER ACRE	INCREASE OVER CHECK							
		1913	1914	1915	1916	1917	1918	1920	Average
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	280 lbs. nitrate of soda	24	−8	109	−13	−8	−28	1	15
3	200 lbs. ammonium sulfate	5	−18	31	1	−45	−29	−21	−11
5	400 lbs. dried blood	−5	2	108	27	23	37	10	29
7	600 lbs. cottonseed meal	10	−6	84	18	40	33	−9	24
9	280 lbs. nitrate or soda 500 lbs. acid phosphate 200 lbs. muriate of potash	42	32	62	99	21	28	47	47
11	200 lbs. ammonium sulfate 500 lbs. acid phosphate 200 lbs. muriate of potash	4	7	34	71	−3	−15	53	22
13	400 lbs. dried blood 500 lbs. acid phosphate 200 lbs. muriate of potash	85	7	85	112	16	1	−4	43
15	600 lbs. cottonseed meal 500 lbs. acid phosphate 200 lbs. muriate of potash	41	41	122	88	2	−36	15	39
17	500 lbs. acid phosphate	1	10	56	82	15	−20	14	23
19	500 lbs. basic slag	14	16	46	14	16	5	12	18
21	300 lbs. rock phosphate	−5	36	96	59	38	16	32	39
23	300 lbs. steamed bone	35	50	48	95	17	20	8	39
25	600 lbs. bone meal 200 lbs. ammonium sulfate 200 lbs. muriate of potash	−17	25	38	109	−19	38	−38	19
27	500 lbs. basic slag 280 lbs. nitrate of soda 200 lbs. muriate of potash	13	111	48	103	−12	−54	23	33
29	300 lbs. rock phosphate 200 lbs. ammonium sulfate 200 lbs. muriate of potash	17	28	−1	23	−48	−42	−67	−15
31	300 lbs. steamed bone 200 lbs. ammonium sulfate 200 lbs. muriate of potash	1	3	60	50	9	−39	−5	11

TABLE 1—*Continued*

PLOT	TREATMENT PER ACRE	INCREASE OVER CHECK							
		1913	1914	1915	1916	1917	1918	1920	Average
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
33	200 lbs. muriate of potash	18	−22	5	−14	8	−2	39	5
35	200 lbs. sulfate of potash	27	34	51	19	−5	4	39	24
37	840 lbs. kainit	25	17	−3	8	−14	−22	−2	−1
39	200 lbs. sulfate of potash 200 lbs. ammonium sulfate 500 lbs. acid phosphate	38	1	67	30	5	96	54	42
41	840 lbs kainit 200 lbs. ammonium sulfate 500 lbs. acid phosphate	21	−1	17	11	6	−3	64	16

At the end of 1915 the vine growth was so great that some pruning was thought advisable and the excessive growth cut from some of the plots. The following list shows which plots were pruned and the weight of the vines removed.

PLOT	POUNDS	PLOT	POUNDS
1	55.50	25	68.00
5	68.30	27	97.50
7	53.75	29	88.00
9	102.00	31	86.00
11	77.00	39	60.50
13	33.75	41	45.00
15	34.00		

Discussion of results

Nitrate of soda started with a good increase the first year, but suffered a loss through insects the second year. The third year it gave an excellent increase, although it had poor staying qualities. The increase dropped to 13 per cent as soon as applications ceased, and then dropped to a loss in 1917 and 1918. It is safe to say that nitrate of soda has a quick action and stimulates vine growth enormously.

Ammonium sulfate, because of its acid reaction, was thought to be ideal for cranberry land at the beginning of the experiments. It was used wherever there was a choice in the complete fertilizers. However, where it was used alone it seemed to injure the vines directly, causing a sickly yellow appearance. This plot did not have vine growth enough to warrant pruning in 1916, nor was the crop especially large. Even after the limestone treatment of 1918,

the plot did not recover. From the action on this plot the writer feels that ammonium sulfate is an unsatisfactory source of nitrogen for cranberry land.

Dried blood started slowly but its action seemed well suited to the cranberry and its average is the best received from any of the sources of nitrogen.

Cottonseed meal acted in a manner somewhat the same as dried blood but on the whole the results were not as satisfactory as those obtained from dried blood.

The results with nitrogen from organic sources check well with those obtained on upland crops, namely that such sources do not return nitrogen pound for pound as effectively as do mineral sources of nitrogen. When we consider that the application of 380 pounds of nitrate of soda is undoubtedly an overdose, we feel that 400 pounds of dried blood must furnish nearly the required amount of nitrogen. In other words, 40 pounds of nitrogen applied in the form of dried blood would give a result nearly equal to the result of a treatment of 20 pounds of nitrogen in the form of nitrate of soda on upland crops, and the same seems to be true on cranberry soil.

Acid-phosphate gave moderate increases from the first, and the average was satisfactory. Its valuable characteristic is its early availability.

Basic slag returned a steady but moderate increase in yield. It was also quick to start.

Rock phosphate was slow to start but gave an excellent crop after the first year. It is probably one of the best sources of phosphoric acid on cranberry soil.

Steamed bone has a small nitrogen content and we must take this into consideration when we compare its effect with that of the phosphatic non-nitrogen treatments. Its increases are rather quick and even, although the average is just equal to the rock phosphate increases.

With the potash the results are rather "hit or miss," but sulfate of potash shows itself to be an even yielder of gains and is probably the best source of potash.

The increases resulting from the complete fertilizer do not lead to any further discussion except that all showed effects of an over fertilization, and that plots 25, 27 and 29 suffered a heavy attack by the cranberry girdler.

In brief, the best sources of nitrogen are nitrate of soda and dried blood; of phosphoric acid, phosphate rock; and of potash, sulfate of potash.

II. AMOUNT AND SOURCES OF NITROGEN

The second problem undertaken was the determination of the optimum amount of nitrogen to be applied to cranberry soil and to compare the effect of nitrogen drawn from the mineral and with that from organic sources. The first study showed that 40 pounds of nitrogen was too much to apply annually, and according to the experience of one of the larger growers 10 pounds was too little. Obviously the optimum was somewhere between these amounts and it was decided to try 20 and 30 pounds.

The treatments and results are recorded in table 2.

Discussion of results

The addition of 140 pounds of sodium nitrate to the acre the first year caused an increase of 37 per cent and the second year of 80 per cent. The treatment of 210 pounds of sodium nitrate first caused an increase of 91 per cent, but the second year it dropped to 58 per cent. This indicates that 30 pounds of nitrogen drawn from sodium nitrate is too great for annual applications, while 20 pounds seems to be nearer the correct amount.

With dried blood the reverse is true, namely, that 30 pounds of nitrogen drawn from the dried blood gives a better yield than 20 pounds. This bears out the conclusion drawn under the first experiment.

TABLE 2

Results of nitrogen experiments on Savannah soil, variety Early Black

PLOT	TREATMENT PER ACRE	NITROGEN APPLIED	1919 Yield per acre	1919 Increase over checks	1920 Yield per acre	1920 Increase over checks
		lbs.	*lbs.*	*per cent*	*lbs.*	*per cent*
F-SB-N 1	Nothing		3320		3680	
F-SB-N 2	140 lbs. sodium nitrate	20	4280	37	6020	80
F-SB-N 3	Nothing		2920		3020	
F-SB-N 4	70 lbs. sodium nitrate; 85 lbs. dried blood	20	4400	48	4920	54
F-SB-N 5	Nothing		3000		3400	
F-SB-N 6	170 lbs. dried blood	20	3200	−2	4320	12
F-SB-N 7	Nothing		3560		4300	
F-SB-N 8	210 lbs. sodium nitrate	30	7320	91	6000	58
F-SB-N 9	Nothing		4100		3300	
F-SB-N 10	105 lbs. sodium nitrate; 127½ lbs. dried blood	30	4660	21	4850	84
F-SB-N 11	Nothing		3160		1980	
F-SB-N 12	255 lbs. dried blood	30	3920	10	2680	52
F-SB-N 13	Nothing		3900		1540	

The increase due to the mixture affording 20 pounds of nitrogen was half way between the increases from the ingredients themselves in 1920, but with a treatment yielding 30 pounds of nitrogen, the increase was more than from either of the ingredients used alone. The reason for this is that the plot receiving nitrate of soda alone has an overdose, while the other plots are receiving less than they can profitably use.

III. CALCIUM CYANAMIDE AS A SOURCE OF NITROGEN

Calcium cyanamide is a relatively new source of nitrogen on the American market and its advantages are little known. In 1919 tests were started to determine its use as a source of nitrogen. The yield was so disappointing at the end of the first year that no new treatment was made in 1920, but the residual effect was recorded. The results are given in table 3.

It seems that the result the first year did not show the full advantage of the application of calcium cyanamide. The distinct loss where calcium cyanamide alone is used the first year is more than made up the second year. The addition of ground limestone does not materially help the treatment.

TABLE 3

Results of tests with calcium cyanamide on Savannah soil, variety Late Howe

PLOT	TREATMENT PER ACRE, 1919 ONLY	1919		1920	
		Yield per acre	Increase over checks	Yield per acre	Increase over checks
		lbs.	*per cent*	*lbs.*	*per cent*
1	Nothing	5680		5653	
2	120 lbs. calcium cyanamide	4900	−17	5653	28
3	Nothing	5900		3147	
4	120 lbs. calcium cyanamide 250 lbs. acid phosphate 220 lbs. sulfate of potash	6000	2	4720	44
5	Nothing	5900		3413	
6	120 lbs. calcium cyanamide 2000 lbs. ground limestone	4740	−20	5113	20
7	Nothing	5900		5113	
8	120 lbs. calcium cyanamide 250 lbs. acid phosphate 220 lbs. sulfate of potash 2000 lbs. ground limestone	6340	6	5387	5
9	Nothing	6040		5113	

IV. THE DETERMINATION OF THE OPTIMUM AMOUNT OF PHOSPHORIC ACID FOR CRANBERRY SOIL

It was recognized that a sufficient amount of phosphoric acid is lacking in most cranberry soils, but the amount that may be applied economically each year is not definitely known. The study here reported is designed to throw light on this problem.

In former investigations reported in this paper phosphoric acid derived from acid phosphate gave immediate returns; on the other hand, phosphoric acid derived from rock phosphate gave no increase until after the first year, but when it began to operate in any marked degree, its results were far better than those of acid phosphate. Rock phosphate, because of its alkaline reaction, did not leave an undesirable residue in the soil and it was the most beneficial of the materials tested. This was used as a basis of the treatments.

As rock phosphate would not become effective before the second year, a treatment was made of an equal amount of phosphoric acid drawn from acid phosphate. This extra treatment was to furnish phosphoric acid for the first year, thc treatment in following years, except on plot 14, to be merely rock phosphate. The treatments and yields are given in table 4.

TABLE 4

Results of phosphate tests, variety Early Black

PLOT	TREATMENT PER ACRE	P_2O_5	1919 Yield per acre	1919 Increase over checks	1920 Yield per acre	1920 Increase over checks
		lbs.	*lbs.*	*per cent*	*lbs.*	*per cent*
1	Nothing		2440		2310	
2	125 lbs. acid phosphate (1919 only)	20				
	75 lbs. phosphate rock	20	2280	−3	2210	12
3	Nothing		2240		1470	
4	250 lbs. acid phosphate (1919 only)	40				
	150 lbs. phosphate rock	40	3720	29	2240	1
5	Nothing		3520		2960	
6	375 lbs. acid phosphate (1919 only)	60				
	225 lbs. phosphate rock	60	3200	−4	3960	40
7	Nothing		3120		2500	
8	500 lbs. acid phosphate (1919 only)	80				
	300 lbs. phosphate rock	80	3580	17	4540	79
9	Nothing		3000		2480	
10	150 lbs. phosphate rock	40	3000	2	2320	6
11	Nothing		2880		2460	
12	150 lbs. soft phosphate rock	40	2840	−2	2220	−2
13	Nothing		2920		2060	
14	250 lbs. acid phosphate	40	3440	11	2160	19
15	Nothing		3360		1560	

Discussion of results

The clear-cut nature of the results is somewhat obscured by the erratic yields on the check plots. However, it is clear that the highest yields and the greatest percentum increase are obtained on the plots receiving the greatest amount of phosphoric acid. The remarkable increase of 79 per cent after treatments of 300 pounds of phosphate rock shows the poverty of the soil with respect to phosphoric acid.

Soft phosphate rock did not show any advantage over the Tennessee phosphate rock.

The experiments are to be carried on through several years in order to obtain a definite knowledge regarding the total quantity of phosphate rock that may be economically added to cranberry soil.

V. BARIUM PHOSPHATE AS A SOURCE OF PHOSPHORIC ACID

Barium phosphate is a new source of phosphoric acid of which we know comparatively little, except that it is a by-product of the iron ore industry. It has been highly recommended at some of the experiment stations, but its use has met with failure at others. It was considered worth a trial on cranberry land, at any rate.

The yields were unsatisfactory after the first treatment and no new treatment was made in 1920. The residual effect on the crop has been recorded. The results are given in table 5.

TABLE 5

Results of tests with barium phosphate, variety Early Black

PLOT	TREATMENT PER ACRE	P_2O_5	1919		1920	
			Yield per acre	Increase over checks	Yield per acre	Increase over checks
		lbs.	*lbs.*	*per cent*	*lbs.*	*per cent*
1	Nothing		2920		2060	
2	250 lbs. acid phosphate	40	3440	11	2160	19
3	Nothing		3360		1560	
4	150 lbs. phosphate rock and 7 per cent barium sulfide (1919 only)	40	3560	8	2240	47
5	Nothing		3160		1540	
6	150 lbs. soft phosphate rock and 7 per cent barium sulfide (1919 only)	40	3240	9	2600	49
7	Nothing		2760		1940	
8	150 lbs. barium sulfide (1919 only)	40	2400	−9	1980	8
9	Nothing		2480		1720	

The use of barium phosphate cannot be recommended from the results of the reported test.

VI. THE OPTIMUM AMOUNT OF A TENTATIVE MIXED FERTILIZER TO BE APPLIED TO SAVANNAH SOIL

The experiment station investigators felt in 1919 that they were ready to publish a tentative formula for a complete fertilizer for Savannah land, and undertook tests to determine as nearly as possible the amount needed for annual applications. The mixture was made up on the basis of the results obtained since 1913, and consists of the following:

	lbs.
Sodium nitrate	75
Dried blood	75
Rock phosphate	300
Sulfate of potash	50

The first year this material was used 300 pounds of acid phosphate was added in order to have phosphate available the year of application. The results of two years' tests are given in table 6.

Plot 8 received what was obviously an overdose of fertilizer in 1919, and the experimenter did not feel justified in repeating the application in 1920. Consequently, the yield from this plot in 1920 was influenced only by the residual effect.

TABLE 6

Results of tests with different amounts of the cranberry fertilizer mixture

PLOT	TREATMENT PER ACRE	1919		1920	
		Yield per acre	Increase over checks	Yield per acre	Increase over checks
		lbs.	*per cent*	*lbs.*	*per cent*
SB-F-C-1	Nothing	3800		1540	
SB-F-C-2	264 lbs. mixture 176 lbs. acid phosphate*	4780	20	2520	61
SB-F-C-3	Nothing	4000		1580	
SB-F-C-4	528 lbs. mixture 352 lbs. acid phosphate	5180	20	2620	68
SB-F-C-5	Nothing	4680		1540	
SB-F-C-6	792 lbs. mixture 528 lbs. acid phosphate	6340	38	3680	82
SB-F-C-7	Nothing	4500		2500	
SB-F-C-8	1056 lbs. mixture 704 lbs. acid phosphate	5200	49	2980†	45
SB-F-C-9	Nothing	2860		1600	

* The acid phosphate was added in 1919 only.

† No fertilizer applied in 1920.

The percentage increases over the checks for both years show that plot 5, receiving 792 pounds of the mixture, gave the best results. From observation in the fall of 1920 this plot was in the best condition to produce a crop in 1921. The yield from the plot receiving 528 pounds of the mixture was very close to that from the one receiving the larger amounts, but the action was somewhat slower. For a bog in a run-down condition like this one, an application of 792 pounds of the mixture will certainly pay good dividends to the grower.

VII. EFFECT OF FERTILIZER APPLICATIONS OTHER THAN ON THE YIELD

Plant-food applications create other differences than in the total yield. Probably the most important difference is in the size of the individual fruits which varies directly with the available plant-food. A single example of this tendency has been selected from the plant-food studies and is fairly typical. The three plots chosen were in a bog badly in need of plant-food; one received nothing, the second received a single application of a complete mixture and the third received a double application of the same mixture. Applications were made in 1917, and the size of the berries was measured in 1918 and 1919. The number of berries required to fill an inspector's cup has been taken as the measurement, and the published data represent the average of three counts made of a lot of berries. The larger numbers of berries indicate the smaller size of the individuals.

TABLE 7

Results showing effect of fertilizer on size of cranberries

PLOT	TREATMENT PER ACRE (SUMMER OF 1917 ONLY)	SIZE OF BERRIES (NUMBER PER MEASURE)	
		1918	1919
1	Nothing	169	123
2	1000 lbs. mixture	128	110
3	2000 lbs. mixture	112	93

TABLE 8

Results showing effects of fertilizer on quality of cranberries

PLOT	TREATMENT PER ACRE	BERRIES AFFECTED BY ROT	
		1918	1919
		per cent	*per cent*
1	Nothing	19	84
2	1000 lbs. mixture	11	2
3	2000 lbs. mixture	21	58

A second difference is in the health of the individual fruits. Table 8 shows the proportion of berries affected by rot in the plots mentioned above.

There is an enormous difference in the amount of rot between the two years recorded, but aside from this, there is a definite difference in the percentage of rot between the individual plots. Plot 2, receiving about the correct amount of plant-food, had the fewest rotten berries both years, while the other plots had a very high proportion of rot.

A third effect of fertilization is in vine growth. This is especially noticeable on the plots receiving nitrogen, where the plants start their growth earlier in spring and stay green later in the fall. The individual leaves are larger and greener than those in the untreated plots.

Indirectly due to the quickened vegetative growth, the treated plots show a smaller number of weeds, a large number of which are crowded out by the well-fed cranberry vines. This is especially true of red root (*Lachnanthis tinctoria* Ell.). Annual weeds are prevented from starting because of lack of space for seed germinating.

DISADVANTAGES OF FERTILIZATION

The more vigorous vine growth caused by fertilizer treatments is very attractive to many insect pests, more particularly the blossom worm and the cranberry girdler. Extensive areas well fertilized have borne large crops four or five years and suddenly stopped through no apparent reason, but the following season dead vines appear here and there and the next year dead spots sometimes a half-acre in extent are common. This is the work of the cranberry girdler.

The most serious injury caused by the blossom worm is its cutting off of the stem of the blossom. It can easily hide in thick vines and so does most of its work there. Both of these pests are easily controlled by submerging the cranberry bog and a careful grower will provide sufficient water supply before he uses fertilizer extensively. Good practice demands that careful inspection be made for these pests, so that the water may be used before they have ruined a crop or a bog.

SUMMARY

The cranberry bog because of its peculiar soil and water conditions requires a special fertilizer mixture.

The most profitable sources of nitrogen appear to be nitrate of soda and dried blood. Sulfate of ammonia is an unsatisfactory source of nitrogen. Acid phosphate and rock phosphate were efficient sources of phosphoric acid. Sulfate of potash and muriate of potash were good sources of potash.

In the production of cranberries 20 pounds of nitrogen drawn from nitrate of soda appears to be as efficient as 30 pounds of nitrogen drawn from dried blood. Twenty pounds of nitrogen from nitrate of soda gave excellent returns on Savannah cranberry land.

Calcium cyanamide as a source of nitrogen is of doubtful value.

Cranberry bogs seem to be very deficient in phosphoric acid, applications as high as 80 pounds of actual phosphoric acid per acre producing large increases in crop.

Cranberry bogs respond readily to applications of the complete fertilizer mixture, from 500 to 800 pounds per acre giving the best results.

Over-fertilization causes excessive vine growth and soft berries, and such a practice makes the vines very susceptible to insect attack.

ACKNOWLEDGMENTS

The writer wishes to take this opportuuity to express his sense of obligation to the Research Committee of the American Cranberry Growers' Association, Messrs. F. S. Chambers, Thomas Durell, H. B. Scammell, and A. F. Freeman; and to Dr. Jacob G. Lipman, Director, and Dr. Thomas J. Headlee, Entomologist, of the New Jersey Agricultural Experiment Stations. The work reported here has been done by the writer in close coöperation with the above-mentioned men.

THE INFLUENCE OF VARYING AMOUNTS OF SULFUR IN THE SOIL, ON CROP YIELDS, HYDROGEN-ION CONCENTRATION, LIME REQUIREMENT AND NITRATE FORMATION[1]

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The senior author has already called attention to the rather rapid oxidation of sulfur when intimately mixed with normal soil, and with McLean and Lint (8, 9) has shown that the phosphoric acid of raw rock phosphate, may be made available by means of the sulfuric acid thus formed. He has also suggested the use of a moderate application of sulfur as a means of combating potato scab through the increased acidity of the soil. However, it was recognized that the use of sulfur in this way might have a pronounced prejudicial influence on the soil reaction and also on nitrate formation and crop yields.

In order, therefore, that more definite information might be secured on these points an experiment was planned in which sulfur was used at the following rates per acre in connection with the growing of barley and soybeans, the latter being grown as a second crop without further treatment.

Plot number	*Pounds per acre*
1a and 1b	200
2a and 2b	500
3a and 3b	1000
4a and 4b	2000
5a and 5b	4000

Inoculated sulfur was used on the *b* series and uninoculated on the *a*'s.

The plots were approximately square and $\frac{1}{200}$ acre in area.

All plots received a uniform application of a complete fertilizer analyzing 4 per cent nitrogen, 10 per cent phosphoric acid, and 3 per cent potash, at the rate of 600 pounds per acre. This was made from dried blood, acid phosphate and muriate of potash. Both sulfur and fertilizer were spread broadcast only a few days before barley was seeded on May 3, 1920.

Germination was fairly uniform, but as the season progressed there was very definite indication of injury with the heavier applications of sulfur. This is shown by the dry weights (grain and straw were not separated) reported in table 1. On the uninoculated section (*a*) there is a gradual decline in the

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yield as the sulfur was increased until with the 4000-pound application the yield is less than half the yield where 200 pounds were used. On the inoculated section the yield was as much with 2000 pounds as with 500 pounds, but with 4000 pounds it was reduced to less than one-half the yield with the 200-pound application. On plots 5a and 5b the blades of the barley plants first turned brown at the tips and then finally died back until at harvest time almost the entire plant was dead. Some weeds and grass grew even after the barley plants had died. The soil of these plots gradually became darker in color than the surrounding soil.

Immediately after the barley was harvested the ground was disked and seeded to soybeans without further sulfur treatment. On plots 1a and 1b germination was about normal, on 2a and 2b not quite so good, on 3a and 3b there were a few scattering plants, and on the remaining plots there was practically no germination. However, beans which fell around the borders of these plots germinated and grew.

TABLE 1

Dry weights of barley and soybeans from sulfur-treated plots

PLOT NUMBER	SULFUR APPLIED PER ACRE	BARLEY (GRAIN AND STRAW)	SOYBEAN HAY
	Uninoculated section		
	lbs.	*lbs.*	*lbs.*
1a	200	2840	963
2a	500	2360	992
3a	1000	2000	690
4a	2000	1710	490
5a	4000	1200	40
	Inoculated section		
1b	200	2460	660
2b	500	2020	826
3b	1000	1730	700
4b	2000	2020	310
5b	4000	1140	32

As the season progressed there was considerable growth of crab-grass and weeds on the less acid plots where the stand of beans was incomplete. At the time of harvesting the beans, plots 4a, 4b, 5a and 5b were barren save a few bunches of crab-grass and smart-weed. It was very evident that certain of the weeds and grasses were more resistant to the unfavorable conditions than the soybeans.

The beans were harvested as hay on October 8. The dry weights are reported in table 1 along with the barley weights. It will be noted that the yield was slightly greater in each case with 500 than with 200 pounds of sulfur. This may be due to the fact that the seeding, which was done by hand, was not entirely uniform. Beginning with the 1000-pound application there

is a rather rapid decline in yield until with 4000 pounds of sulfur there were only 32 and 40 pounds, respectively, of dry matter per acre, which consisted of grass and weeds.

HYDROGEN-ION CONCENTRATION OF THE SOIL SOLUTION

In order that some information might be gained with reference to the rapidity with which the sulfur was oxidized, it was decided to make hydrogen-ion concentration determinations at intervals of two weeks. Since no check plots were provided in the seeded portion two unseeded plots lying beside the sulfured plots were taken as check plots for the soil work.

TABLE 2

Hydrogen-ion concentration expressed as pH values—sulfur-treated plots, 1920

PLOT NUMBER	MAY 17	JUNE 1	JUNE 14	JUNE 28	JULY 12	JULY 26	AUGUST 9	AUGUST 24	SEPTEMBER 9	SEPTEMBER 23	OCTOBER 7	OCTOBER 21	NOVEMBER 4	NOVEMBER 18	AVERAGE
	pH	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
1, check	5.6	5.6	5.6	5.6	5.7	5.7	5.8	5.9	6.2	6.3	6.2	6.3	6.2	5.9	5.9
2, check	5.8	5.8	5.8	5.8	5.9	5.9	6.0	6.0	6.2	6.4	6.3	6.3	6.2	6.0	6.0
1a	5.8	5.8	5.8	5.8	5.8	5.8	5.9	6.0	6.2	6.1	6.0	6.1	6.1	5.8	5.9
2a	5.6	5.5	5.5	5.7	5.8	5.5	5.6	5.4	5.4	5.8	5.9	5.0*	5.9	5.8	5.6
3a	5.6	5.6	5.5	5.0	4.8	4.8	4.8	4.5	4.6	4.8	4.6	4.9	4.6	4.9	4.9
4a	5.6	5.6	5.6	4.8	4.4	4.0	4.1	4.2	4.0	3.8	3.9	4.0	3.9	3.9	4.4
5a	5.6	5.5	4.9	4.5	3.9	3.9	3.5	3.6	3.8	3.6	3.8	3.8	3.8	3.9	4.2
1b	5.6	5.6	5.6	5.6	5.9	5.8	5.8	5.8	6.1	6.0	6.0	6.1	6.0	5.9	5.8
2b	5.6	5.6	5.6	5.7	5.8	5.8	5.8	5.6	5.8	5.8	5.8	5.9	5.8	6.0	5.8
3b	5.6	5.6	5.6	5.0	4.6	4.7	4.7	4.7	4.7	4.7	4.6	4.4	4.3	4.4	4.8
4b	5.6	5.5	5.4	4.7	4.1	3.9	3.6	3.8	3.9	3.9	3.8	3.8	4.0	4.2	4.3
5b	5.6	5.5	5.0	4.3	3.8	3.9	3.6	3.8	3.8	3.7	3.9	3.9	3.9	3.9	4.2

* Omitted from average.

Sampling was begun May 17, 2 weeks from the date of seeding the barley. Five or more cores of about 1 inch diameter were taken from each plot, cutting to the depth of $6\frac{2}{3}$ inches, and these were combined to make one sample for the plot. The samples were immediately taken to the laboratory where they were air-dried and prepared for analysis without grinding. The hydrogen-ion determinations were made by the colorimetric method, the double-tube standard described by Gillespie (5) being used. The results of these determinations are reported in table 2. The check plots ran fairly constant around 5.9 to 6.0 throughout the season, with a slight tendency to become less acid toward the end of the season. Check 1 was slightly more acid than check 2 during the first half of the season. Plots 1a and 1b (200 pounds of sulfur) gave readings quite close to the check plots and here also there was a tendency toward the less acid condition near the end of the season.

On an average 2a shows a slightly higher acidity than 2b, but for a number of the samplings the readings are identical. The point of highest acidity for these plots was reached August 24 (16th week). From this time on, there was a tendency toward a decline in acidity until November 18 when the readings were 5.8 and 6.0, respectively, with an average of 5.6 and 5.8.[2]

Beginning about the sixth to the eighth week plots 3a and 3b (1000 pounds of sulfur) show an increase in acidity. For 3a the maximum acidity occurred

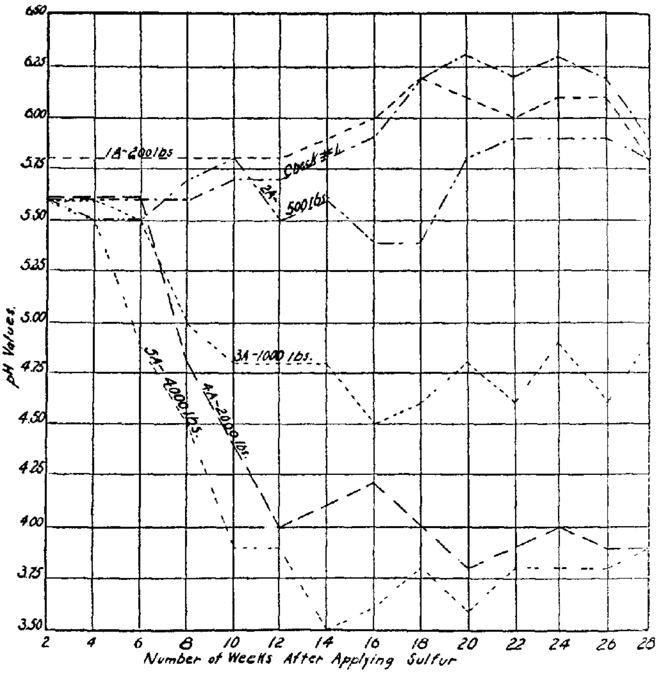

FIG. 1. HYDROGEN-ION CONCENTRATION (pH VALUES) WITH VARYING AMOUNTS OF UNINOCULATED SULFUR

August 24 and for 3b on November 4. The average for the period was 4.9 and 4.8, respectively.

A decided increase in acidity is noted for 4a and 4b on June 28 (eighth week), though 4b showed slight increase the fourth and sixth weeks. The maximum for both plots was reached August 9 when the pH value was 3.6.

[2] A reading of 5.0 for 2a on October 21 has been omitted from the average, since no explanation was found for a reading which differs so widely from those for samples taken two weeks earlier and two weeks later.

On November 18 the readings were 3.9 and 4.2, respectively, with an average of 4.4 and 4.3

The maximum acidity for 5a and 5b also occurred on August 9 when the readings were 3.5 and 3.6, respectively, with a period average of 4.2 and 4.2.

As previously pointed out these plots and also 4a and 4b were so strongly acid that the soybeans were practically a failure.

These differences in reaction are clearly brought out by the graphs in figures 1 and 2.

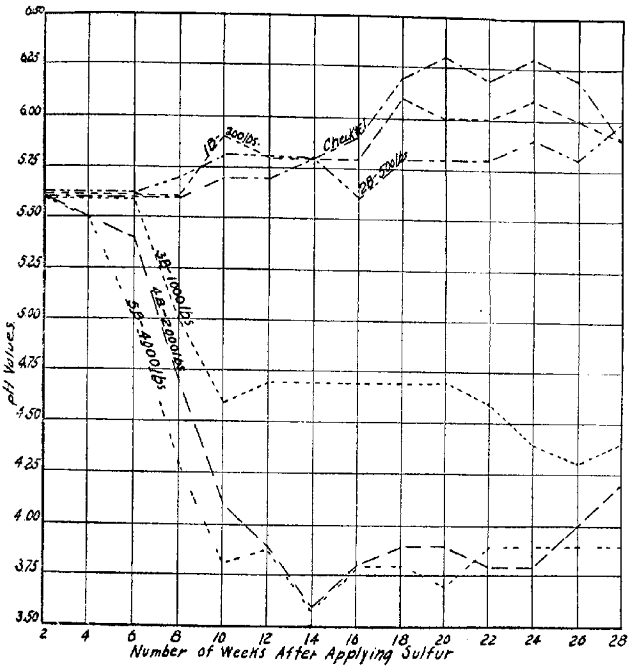

FIG. 2. HYDROGEN-ION CONCENTRATION (pH VALUES) WITH VARYING AMOUNTS OF INOCULATED SULFUR

LIME REQUIREMENT COMPARED WITH HYDROGEN-ION CONCENTRATION

Lime-requirement determinations (revised Veitch method) (14) were made on samples from section *a* collected May 17, June 28, August 9, September 23 and November 4, these dates being at intervals of about 6 weeks.

In table 3 these results are compared with the pH values obtained on the same samples. From the table it will be noted that the lime requirement was fairly constant—800 to 1000 pounds—for all of the plots on May 17,

about two weeks from the date of applying sulfur. With only two exceptions (plot 1a) the requirement did not vary greatly from these figures for the check plot and plots 1a and 2a, throughout the season.

On June 28, however, there was a distinct increase beginning with plot 3a (1000 pounds of sulfur per acre), the lime requirement being 1400 pounds. For 4a and 5a the requirement on this date was 2800 pounds.

This rather sharp rise in lime requirement for 3a and 4a is noted again on August 9, September 23 and November 4, and for 5a on August 9, this latter being the highest lime requirement noted during the period under consideration.

It is of especial interest to note that 500 pounds of sulfur did not materially increase the lime requirement over that of the check plot. It did, however, result in some increase in the pH value. In this connection it should be remembered that this land was in crops that were not cultivated. The only

TABLE 3

Hydrogen-ion concentration and lime requirement of soils from plots that have received differen quantities of sulfur

PLOT NUMBER	SULFUR TREATMENT	MAY 17		JUNE 28		AUGUST 9		SEPTEMBER 23		NOVEMBER 4		AVERAGE	
		pH value	Lime requirement	pH value	Lime requirement	pH value	Lime requirement	pH value	Lime requirement	pH value	Lime requirement	pH value	Lime requirement
	lbs.		*lbs.*		*lbs.*		*lbs.*		*lbs.*		*lbs.*		*lbs.*
1, check	0	5.6	1000	5.6	800	5.8	800	6.3	800	6.2	800	5.90	840
1a	200	5.8	800	5.8	800	5.9	600	6.1	200	6.1	200	5.94	520
2a	500	5.6	800	5.7	600	5.6	1000	5.8	1000	5.9	900	5.70	860
3a	1000	5.6	800	5.0	1400	4.8	2200	4.8	2000	4.6	3000	4.96	1880
4a	2000	5.6	800	4.8	2800	4.1	4600	3.8	6000	3.9	6400	4.44	4120
5a	4000	5.6	1000	4.5	2800	3.5	7600	3.6	6600	3.8	6000	4.20	4800

stirring that it received after the sulfur was harrowed in, was the disking preparatory to seeding the soybeans. Had the land been in cultivated crops it is quite possible that the 500-pound application of sulfur might have influenced the hydrogen-ion concentration and the lime requirement to a greater extent.

Likewise on a different type of soil or with a higher initial pH value the results might have been different.

With soils having, in most cases, a somewhat higher initial pH value than the soil in question, Martin (10) found that 300 to 600 pounds of sulfur per acre did considerably increase the hydrogen-ion concentration of the soil solution as compared with plots that received no sulfur.

The 1000, 2000 and 4000-pound applications of sulfur did make very sharp increases in both the pH values and the lime requirement after the sulfur had been in the ground for about 6 weeks.

The principles involved in the two methods of reporting the soil reaction differ so widely that it is not practicable to attempt a direct comparison. The junior authors (2), however, have already called attention to the fact that an increase in hydrogen-ion concentration, as shown by the pH value, is usually paralleled by some increase in lime requirement.

It is quite evident from the work reported here that a determination of pH values in many cases will enable one to make an approximate forecast of what the lime requirement will be.

For example, in connection with the soils here considered there would seem to be justification for the statement that a pH value of about 5.0 to 4.6 means a lime requirement ranging between 2000 and 3000 pounds. This it must be admitted is a rather wide range, but it may not be much farther out of the way than results secured by some of the methods now in vogue.

The average lime requirement for the five samples of plot 2a is 860, which is practically the same as the average for the check plot. For 3a (1000 pounds of sulfur) it is 1880 pounds, for 4a, 4120 pounds, and for 5a, 4800 pounds. The corresponding pH values are 5.0, 4.4 and 4.2.

NITRATE DETERMINATIONS

The samples collected for hydrogen-ion determinations served also for nitrate determinations, which were likewise made at intervals of two weeks. In a consideration of this subject it must be remembered that nitrates are subject to rather wide variations during the season, depending upon several factors, among which may be mentioned rainfall, temperature, the growing crop, the quantity of decomposing organic matter that is present, and possibly in some cases, to soil reaction. Therefore, when the nitrate content becomes less in the presence of a growing crop it is not necessarily an indication that nitrates are not being formed, or are being leached out by percolating rain-water. It may simply mean that they are being utilized by the crop more rapidly than at some other time when a higher concentration was noted.

In determining the nitrates the colorimetric method described by Schreiner and Failyer (12) was used.

As already explained the check plots were not true checks, since no crop was grown on them, nor was any fertilizer used on them. An attempt was made to keep them bare, but at times the weeds and grass made some headway. For these reasons, therefore, the readings obtained from these checks cannot have the value of true checks, but they seem to be of sufficient worth to warrant their inclusion in the report.

Between the fourth and sixteenth weeks there is indication of a considerable accumulation of nitrates on these checks. After this time the concentration is rather low, with the exception of check 2 for the twenty-sixth week (November 4). For all the sulfur-treated plots there is a gradual downward trend in nitrate content until the twelfth week, when the average was less

than one part per million. At this time the check plots gave a reading of 5.6 and 4.0 parts of nitrates per million.

The rainfall at the New Jersey station for the months of June, July and August, 1920, was above the last preceding 10-year average for these months, and it might be claimed that the low nitrate content on the sulfur-treated plots during this period was due to the leaching out of the nitrates by percolating rain-water. Such a claim, however, does not seem logical in view of the rather high content of nitrates found on the check plots during these months. These received no nitrogenous fertilizer, and certainly if the depres-

TABLE 4

Nitrates (NO_3) expressed as parts per million in sulfur-treated plots, 1920

PLOT NUMBER	MAY 17	JUNE 1	JUNE 14	JUNE 28	JULY 12	JULY 26	AUGUST 9	AUGUST 24	SEPTEMBER 9	SEPTEMBER 23	OCTOBER 7	OCTOBER 21	NOVEMBER 4	NOVEMBER 18	AVERAGE
1, check	3.7	7.5	5.8	17.7	11.1	5.6	14.9	10.2	2.3	1.4	1.5	2.0	1.4	2.6	6.3
2, check	3.0	3.7	5.9	9.2	11.9	4.0	8.8	4.4	1.5	1.3	1.8	2.1	14.1	1.8	5.3
1a	10.8	4.6	1.2	2.0	1.2	1.1	2.2	1.9	1.2	1.3	0.9	1.2	1.3	2.1	2.4
2a	3.8	3.5	1.2	1.8	1.2	1.0	3.5	1.8	1.4	1.5	1.2	1.1	1.5	4.4	2.1
3a	3.5	4.1	1.0	1.6	1.1	0.8	2.3	2.1	2.6	4.0	3.3	4.7	2.4	5.3	2.8
4a	4.1	2.4	1.1	1.2	1.0	0.9	1.2	1.8	5.8	4.4	4.0	5.9	4.0	6.6	3.2
5a	6.2	3.4	0.9	1.1	1.2	0.8	2.0	1.8	4.2	4.0	4.4	4.2	4.4	4.4	3.1
Average 1a–5a.....	5.7	3.6	1.1	1.5	1.1	0.9	2.2	1.9	3.0	3.0	3.0	3.4	2.7	4.6	
1b	4.7	1.8	1.1	1.5	1.1	0.9	3.3	1.5	1.4	1.2	1.2	1.5	1.6	2.2	1.8
2b	3.4	1.9	1.1	1.6	1.1	0.8	2.9	1.9	1.0	1.6	1.5	1.6	2.4	2.2	1.7
3b	2.5	1.8	0.8	1.1	0.9	0.7	3.3	1.8	2.4	2.1	3.0	2.2	1.9	3.9	2.1
4b	4.6	1.9	1.3	1.1	0.9	0.8	1.8	1.3	2.7	4.4	4.4	2.7	7.3	8.8	3.2
5b	4.7	2.8	1.3	1.2	0.9	1.0	1.8	2.1	2.9	5.1	3.1	3.7	16.4*	2.2	2.3
Average 1b–5b.....	4.0	2.0	1.1	1.3	1.0	0.8	2.6	1.7	2.1	3.0	2.6	2.3	3.3	4.0	

* Omitted from average.

sion had been caused by the leaching out of the nitrates these plots should have shown at least as low a concentration as the sulfur-treated plots which did receive a nitrogenous fertilizer.

It seems evident, therefore, that the low nitrate concentration noted on the sulfur-treated plots during July and August must be attributed to the removal of nitrates by the growing crop, for the barley (and weeds which were mixed with it), did make a fair growth on plots 1 to 3 of each section and some growth on plots 4 and 5. It is of interest to note that the low point in this downward trend of nitrate concentration was reached near the time of harvesting the barley. From July 26, when the average concentration of the treated plots

was less than one part per million, to August 9, there was a distinct increase in nitrates for all plots, including the checks. This would seem to indicate a period of rather rapid nitrification, but the increase was no doubt due in part to the fact that the soybeans (and weeds which grew with them) had not yet made very much growth.

From August 9 to 24 there was a decrease in nitrate concentration on nearly all the plots, the average at this time being less than two parts per million. From August 24 to November 4, plots 1 and 2 of each section showed a fairly uniform concentration, the amount varying between one and two parts per million. During this same period plots 3 to 5, inclusive, on both sections showed a distinctly higher concentration. This higher concentration was well maintained until the last sampling on November 18, at which time the work was stopped on account of the difficulty of securing samples during freezing weather.

This increase of nitrates on the plots that received from 1000 to 4000 pounds of sulfur per acre was no doubt due to the fact that they were supporting very little plant growth during this period.

This gradual increase of nitrate formation under such acid conditions would seem to indicate that nitrification was not seriously hindered on account of the rather extreme acid condition of the soil.

This is contrary to the generally accepted opinion and also to results secured by Hall and his associates (6), who found that nitrification was much hindered on permanent grass lands which were highly acid.

On the other hand, it is in accord with results secured at the Pennsylvania station where Brown and MacIntire (3) and later White (15) showed that frequently nitrification proceeded as rapidly on soils having a high lime requirement as on those having a low requirement.

It is further corroborated by results secured by the junior authors (unpublished data) during the summer of 1920. Nitrate determinations were made at intervals of two weeks beginning May 29 and continuing until November 25 on samples from field plot 11A, which has received annual dressings of ammonium sulfate without lime since 1908, and now has a lime requirement of 2500 to 3000 pounds of CaO for the plowed acre, and from field plot 11B which receives the same ammonium-sulfate treatment, but is limed at intervals of 5 years and is now about neutral in reaction.

The former gave an average of 16.22 parts of nitrate per million with a range of 1.11 parts on August 9 and 46 parts on June 28, and the latter an average of 6.29 parts per million and a range of 2.21 parts on June 14 and 17.68 parts on May 29. In this connection it must be explained that plot 11B yielded a much larger crop than 11A, and therefore utilized more of the soil nitrates, but at the same time it is evident that nitrification was proceeding freely, for the rainfall during the period was above normal.

Fraps (4) studied the nitrifying power of acid and non-acid soils, and found that some acid soils showed high nitrifying power, while others showed a low

nitrifying power. The work was carried out on 500 gm. of soil in percolators. He concluded that "acid soils nitrify slightly less, on an average, than non-acid soils with a low lime content. Some acid soils do not nitrify at all, or have a low nitrification, while other acid soils have a high nitrification."

Noyes and Conner (11) made nitrification studies on five typical acid soils and concluded that "the amount of nitrates present and the nitrifying power of the untreated acid soils varied with the organic matter and total nitrogen rather than with the soil acidity."

Hoagland (7) found as high as 100 to 3000 parts per million of nitrates (dry-soil basis) in peat soils which gave pH values as low as 6.0 to 4.5.

Temple (13) added 1 gm. of tartaric acid, or its equivalent of citric acid, along with ammonium tartrate, to 200 gm. of soil and found that while the amount of nitrites and nitrates formed was not as large as in soils to which ammonium tartrate and calcium carbonate had been added, the amount formed was decided.

Ames and Richmond (1) found that the oxidation of sulfur in soils not well supplied with bases, resulted in depressing the activites of nitrifying organisms.

SUMMARY

Barley and soybeans (residual crop) were grown on plots to which inoculated and uninoculated sulfur had been applied at the rate of 200, 500, 1000, 2000 and 4000 pounds per acre.

The barley showed fair germination on all the plots, but as the season progressed there was evidence of injury with 1000 pounds and over of sulfur. Before harvest the plants on the plots that received 4000 pounds of sulfur had practically all been killed.

With 200 and 500 pounds of sulfur germination and growth of the soybeans appeared to be about normal. With 1000 pounds and over germination was very materially depressed, there being very few plants on the plots that received 2000 and 4000 pounds of sulfur.

Determinations of hydrogen-ion concentration and nitrates were made on samples of soil from all plots collected at intervals of about two weeks beginning May 17 and continuing until November 18.

Applications of 200 and 500 pounds of sulfur did not very materially change the hydrogen-ion concentration throughout the season. Applications of 1000 to 4000 pounds caused a decided increase in concentration after the fourth to eighth weeks. In most cases the highest concentration was reached toward the last of July or early in August. The minimum pH value noted was 3.5 on plot 5a, and 3.6 on plot 5b, both of these readings being obtained August 9.

Lime-requirement determinations were made on a limited number of the samples collected at intervals of about 6 weeks. From data thus obtained it was found that the 200 and 500-pound applications of sulfur did not materially influence the lime requirement; the heavier applications did cause very decided increases in lime requirement. It was noted that in most cases an

increase in hydrogen-ion concentration was accompanied by an increase in lime requirement, but there is not a direct correlation. The work seems to indicate the possibility of an approximate forecasting of lime requirement from the hydrogen-ion concentration, though the relationship differs with different soils and under different treatments.

The nitrates varied rather widely, apparently depending upon the crop growth. On an average they were higher on the check plots than on the treated plots, but in this connection it must be remembered that these check plots did not grow a crop, but were kept nearly free from vegetation.

The lowest concentration of nitrates was noted near the end of July, soon after the barley had been harvested.

Nitrates were found in considerable quantities in samples from those plots that showed the highest hydrogen-ion concentration. This seems to indicate rather definitely that nitrification is not necessarily inhibited by a highly acid condition of the soil.

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METHODS OF STUDYING THE CONCENTRATION AND COMPOSITION OF THE SOIL SOLUTION[1]

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A more exact knowledge of the soil solution is desirable for the study of many of the problems of soil fertility and related subjects. The purpose of the present investigation was to study some of the methods which have been used in determining the concentration and composition of the soil solution and to compare the results obtained by the different methods.

The methods which have been used may be classified into groups as follows:

(a) Methods involving extraction with comparatively large amounts of water.

(b) Methods which aim to obtain the true soil solution.

(c) Methods which aim to measure the concentration of the soil solution directly in the soil.

The water-extraction method has been widely used and possesses many advantages. The greatest criticism of the method is that the addition of a large quantity of water alters the equilibrium in the soil. It undoubtedly has a solvent effect and may also cause a precipitation of some of the material in solution due to an alteration in the nature of the solvent. The quantity of salts obtained depends upon a number of factors. Mitscherlich (17) has shown the effect of the CO_2 content of the water, the time of extraction, and the ratio of soil to water on the quantity of material extracted. The procedure is arbitrary, but results obtained by several investigators indicate that the usual 1:5 extraction gives an approximate measure of the salt content of the soil solution.

Because of the lack of knowledge as to whether or not the water extraction gives a good quantitative measurement of the salts in the soil, there have been several methods proposed for obtaining the true soil solution.

Ramann, Marz and Bauer (21) have obtained the soil solution by the use of a hydraulic press. They applied a pressure of about 4,000 pounds to the square inch. Lipman (16) also used the pressure method, applying a maxi-

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mum pressure of 53,000 pounds to the square inch. The method is of limited value for it is only applicable to finer-textured soils at a rather high moisture content and requires a complicated apparatus. The criticism has been made by Northrup (19) that the application of high pressures such as were used by Lipman would alter the physico-chemical equilibrium in the soil and as a result the true soil solution would not be secured.

The centrifuge method of Briggs and McLane (23) and the artificial root method of Briggs and McCall (8), in which suction is used, may give the true soil solution. However, these methods are only applicable to soils at high moisture contents and only small amounts of the solution are obtained.

The displacement method was first used by Schloesing (22). He used water colored with carmine to displace the soil solution and obtained considerable amounts which were used for analytical purposes. Gola (9) used water as the displacing liquid in his studies on the concentration of the soil solution. Ischerekov (15) used ethyl alcohol as the displacing liquid and obtained results which indicate that the displaced solution is the true soil solution in an unaltered condition. Moist soil was packed in a glass tube which had a piece of linen tied over the bottom. After placing alcohol on top of the soil column the soil solution soon began to drop from the bottom of the tube. He reports results which indicate that the successive portions of the displaced solution are of the same composition, and that the concentration of the soil solution is inversely proportional to the moisture content of the soil.

Van Suchtelen (25) modified Ischerekov's method by using paraffin oil as the displacing liquid and applying suction to hasten displacement. Morgan (18) used a combination of the pressure and displacement methods in which a heavy oil was used as the displacing liquid and applied pressures of about 500 pounds to the square inch to force the oil into the packed soil. Large quantities of the soil solution were thus obtained. The method is open to the objection that it requires a complicated apparatus and the use of a heavy oil makes it uncleanly.

The writer has been unable to find any reference in the literature in which a comparison was made of the results obtained by the displacement and water-extraction methods.

Several methods have been suggested for determining the concentration of the soil solution directly in the soil. Among the first of these was the measurement of the salt content by electrical conductance (10). The method is of some use in determinations of alkali in soils but the results are affected by the texture, organic matter, carbonates, and the moisture content of the soil. It has not proved of any great value in investigational work.

Bouyoucos and McCool (5, 6) have advanced the freezing-point method as a means of determining the concentration of the soil solution directly in the soil, and as a means of measuring the absolute salt content of the soil (7). The results obtained by this method will be discussed in the latter portion of this paper.

In the present investigation a study was made of the displacement and freezing-point methods. The results obtained by displacement are compared with those obtained by the freezing-point and water-extraction methods.

THE DISPLACEMENT METHOD

Description of the method and procedure

The method consists of packing the moist soil in a cylinder provided with an outlet at the bottom. The displacing liquid is then poured on top of the soil column and as it penetrates the soil it displaces some of the soil solution which forms a zone of saturation below the displacing liquid. This zone increases in depth as it is continually forced downward by the pressure of the liquid above. When the saturated zone reaches the bottom of the soil column the clear soil solution, free of alcohol, drops from the soil as gravitational water.

The only apparatus required is a cylinder in which to pack the soil. The diameter of the soil column very largely determines the rate at which the soil solution will be obtained. The height of the soil column likewise determines the time required for displacement. These two factors must be considered in the selection of the cylinder to be used.

Three different-sized cylinders were used in the present investigation. Brass soil tubes 2 inches in diameter and 9 or 12 inches in depth were used in the preliminary work and when only small amounts of the soil solution were desired. Large brass soil tubes 3 inches in diameter were used for securing larger quantities of the solution and in studying the composition of successive portions of the displaced solution. These tubes were made in 6-inch sections and three or four sections were generally used. The bottom section was provided with a false screen bottom and a small outlet. Glass percolators, $2\frac{3}{4}$ inches in diameter at the top and 15 inches deep, were used in most of the work. The bottom of the percolator was fitted with a small one-hole stopper. A small quantity of coarse quartz sand was placed in the percolator before adding the soil.

The soil was packed in the tubes by means of a short wooden rod. No difficulty was experienced in obtaining uniform packing. The degree of packing is determined by the kind of soil and its moisture content. Sandy soils were packed as firmly as possible at all moisture contents. Peats also may be packed firmly, for there is no danger of puddling the soil and rendering it impervious to the displacing liquid. With the heavier classes of soil care must be taken to prevent puddling during the packing, in which case the rate of displacement is exceedingly slow or entirely prevented. For this reason it is best to use the heavier soils at a moisture content somewhat below the optimum for plant growth. Under proper moisture conditions the soil should not stick together too readily when squeezed in the hand but should be rather granular and easily worked. Miami silt loam was best used at a moisture

content of about 20 per cent and when properly packed had an apparent specific gravity of 1.50 to 1.60. After a little experience one can readily determine the proper degree of packing for any soil at a given moisture content.

After packing, the cylinders were placed in ring stands and the displacing liquid added and maintained at a depth of 2 to 3 inches.

The time required for displacement varied widely, depending on the moisture content of the soil, the degree of packing, the soil type and the height of the soil column. In most cases it is possible to complete the displacement in 12 hours if the height of the soil column is not over 12 or 14 inches. The displacement may be stopped at any time by removing the layer of the displacing liquid on top of the soil column. In some cases the displacement was started in the evening and completed the next day. When silt loam soils were very firmly packed it sometimes required several days to complete the displacement.

In the water-extraction method the extracts were made by adding the desired amount of distilled water to the soil in a large mortar and stirring for 3 minutes. After settling 12 minutes the suspension was filtered through Pasteur-Chamberland filters.

In the displacement method filtration is unnecessary and total salts were determined by evaporating 25 cc. of the soil solution in a platinum crucible. In the water-extraction method larger quantities were evaporated in platinum dishes. After evaporation the crucibles and dishes were placed in an electric oven at 105°C. for 12 hours. The weight of the residue represented total salts before ignition. The crucible and contents were then ignited to constant weight to determine the total salts after ignition.

Nitrates were determined colorimetrically by the phenoldisulfonic acid method.

Calcium was determined volumetrically by titration of the oxalate with potassium permanganate.

Freezing-point determinations were made in the usual manner with a Beckman thermometer.

The effect of different displacing liquids on the time and percentage displacement

Water, ethyl alcohol, and paraffin oil were the liquids employed by previous investigators who used the displacement method. It seemed desirable to try other liquids to determine which would give the most complete and rapid displacement. In the preliminary work it was found that liquids which were non-miscible with water such as benzene, kerosene, ligroin, and ethyl acetate would not satisfactorily displace the soil solution. These liquids passed through the soil in practically an unaltered condition and displaced practically none of the soil solution. To use these liquids it would be necessary to pack the soil more and use pressure, as was done by Morgan (18).

The four liquids studied were ethyl alcohol, methyl alcohol, acetone and water. Miami silt loam at a moisture content of 21 per cent was packed in

four 3-inch brass cylinders, care being taken to obtain uniform packing. The degree of packing in these cylinders was not great enough to obtain the most complete displacement. The time which elapsed between the addition of the displacing liquid and the appearance of the first drop of the soil solution was recorded. The volume of the water in the soil being known, the percentage displaced was readily calculated from the volume of the solution obtained. In order to detect the first appearance of the displacing liquid in the soil solution a freezing-point determination was used. The freezing point of successive portions of the solution was determined. As soon as the displacing liquid appeared in the solution the freezing point was appreciably changed. The appearance of ethyl alcohol and acetone was further confirmed by qualitative tests.

Table 1 shows the effect of different liquids on the time and percentage displacement. The viscosity of the liquids also is given in the table, since viscosity is one of the main factors influencing the time and percentage displacement. The less viscous displacing liquids pass through the pore spaces of

TABLE 1

The time and percentage displacement of the soil solution from Miami silt loam by different liquids

DISPLACING LIQUID	VISCOSITY IN C. G. S. UNITS AT 20°C.	TIME TO THE FIRST DROP	DISPLACEMENT
		hrs.	*per cent*
Acetone	0.00334	2	12.0
Methyl alcohol	0.00591	3½	24.0
Water	0.01006	4	20.0
Ethyl alcohol	0.01190	4½	36.0

the soil more readily than do the more viscous liquids. This causes them to mix with a greater portion of the soil solution.

These and similar results obtained with other soils indicate that ethyl alcohol is the most satisfactory displacing liquid. It gives a more complete displacement than the other liquids used and it is very easy to test for its appearance in the displaced solution by means of the iodoform reaction.

Water is a fairly satisfactory displacing liquid but it mixes more with the soil solution and does not give as complete a displacement as does ethyl alcohol. If water is used some NaCl should be added making it possible to determine when the displacing solution appears by testing with silver nitrate.

Acetone is not at all satisfactory for it has too low a viscosity and therefore passes through the soil too readily, giving a very low percentage displacement. Methyl alcohol possesses no marked advantage over water and is not as good as ethyl alcohol.

Before ethyl alcohol was selected for subsequent work, additional experiments were made to determine the percentage displacement that would ordinarily be obtained by its use. The percentage displacement depends upon

several factors. The higher the soil column and the more compact the soil, the greater will be the percentage of the soil solution displaced. A high moisture content also tends to produce a high percentage displacement. However, these same factors determine very largely the time required for displacement, and the time element should not be made too great.

The experiments indicated that it was practicable with most soils to obtain from 35 to 45 per cent of the soil solution by displacement with ethyl alcohol. This amount may be secured without the time element becoming very objectionable. It is possible to displace a much greater percentage than this. Using a silt loam soil at a moisture content of 23.3 per cent, a 75.6 per cent displacement was secured. Ischerekov (15) reports that with a soil at saturation it is possible to displace 95 per cent of the soil solution.

The concentration of the soil solution obtained by the use of different displacing liquids

A consideration of the mechanics of displacement leads to the conclusion that the soil solution obtained is in all cases really displaced by the soil solution itself. A zone, in which the soil is saturated with the soil solution, soon forms immediately below the displacing liquid after it is added. After the formation of this zone the only function of the displacing liquid is to give pressure and cause a downward movement of the saturated zone. Therefore, the displacing liquid should not affect the concentration of the solution obtained. The question of the influence of the displacing liquid on the concentration of the solution was studied experimentally, using the solutions secured from Miami silt loam by the different liquids shown in table 1. The results are recorded in table 2 and confirm the conclusions reached by a theoretical consideration of the question.

TABLE 2

The concentration of the soil solution obtained with different displacing liquids

DISPLACING LIQUID	TOTAL SALTS IN SOLUTION		FREEZING-POINT DEPRESSION OF SOLUTION
	Before ignition	After ignition	
	p. p. m.	*p. p. m.*	°C.
Acetone	655	248	0.020
Methyl alcohol	649	246	0.019
Water	670	232	0.020
Ethyl alcohol	660	248	0.019

Composition of successive portions of the displaced solution

In displacement the soil solution moves through the soil. The first portions move only a short distance before they drop from the soil, while the last portion may pass through a soil column of considerable height. The question

at once arises as to whether or not the movement of the soil solution through the soil alters its composition. If it does, successive portions of the displaced solution would not be of the same composition. If they are not of the same composition the method probably would be of little value.

In a well mixed soil the solution in all portions is probably of the same composition. The readily soluble salts are in solution and this solution is in equilibrium with the surrounding solid material. As the solution is displaced and passes downward it comes in contact with more solution of the same composition and concentration and with solid material of the same nature as that from which it was displaced. Therefore, the point of equilibrium should not change and the composition of the solution should not be altered by its passage through a column of soil which has been well mixed before it is packed in the cylinder. Hoagland, Martin and Stewart (14) have shown that a water extract of a soil when concentrated and allowed to percolate through another portion of the same soil does not alter much in its composition. It is therefore probable that the composition of the true soil solution would not be changed in passing through a soil column.

TABLE 3

The freezing-point depression and total salts in successive portions of the soil solution from Miami silt loam

PORTION	FREEZING-POINT DEPRESSION	TOTAL SALTS IN SOLUTION	
		Before ignition	After ignition
	°C.	*p. p. m.*	*p. p. m.*
1	0.024	480*	200*
2	0.023		
3	0.024	352	168
4	0.025		
5	0.024	360	176
6	0.022		
7	0.022	324	172
8	0.025		
9	0.076†	344	180
10	0.098†		
11	0.163†	344	176
12	0.309†		
13	0.444†	496*	256*
14	0.689†		

* High results due to colloidal material.

† High results due to alcohol in the solution.

If the soil solution has a solvent effect on the soil particles during its passage through the soil, the last portions would be more concentrated than the first. To determine whether or not successive portions are of the same composition, as indicated by a determination of the freezing point and total salts, a 3-inch brass cylinder was filled with Miami silt loam containing 22 per cent moisture.

The height of the soil column was 22 inches and 35.3 per cent of the soil solution was obtained. During displacement, successive portions were secured and the freezing point determined. Then portions 1 and 2, 3 and 4, 5 and 6, etc., were combined and the total salts determined in these larger portions. Table 3 presents the results.

The first portion contained a small amount of colloidal material which caused a high result for total salts in that portion. Small amounts of alcohol began to come through in the ninth portion, as is indicated by the freezing-point depression. However, the amount was so small that the total salts were not affected until the thirteenth portion. Then the solution became turbid due to colloidal material.

The results show that successive portions are of the same composition. Results have also been obtained showing that successive portions contain the same amount of nitrate nitrogen. It is probable that a complete analysis of the successive portions would prove that they were of the same composition in all respects. These results agree with those obtained by Ischerekov (15) and Schloesing (22). Ischerekov determined total salts and Schloesing determined nitrates.

The concentration of the soil solution at different moisture contents

In most soils the soil solution is very dilute. All readily soluble material is in solution even at low moisture contents. The solution is only saturated in respect to those minerals which are comparatively insoluble and have a low rate of solubility. Therefore, the addition of a small amount of water should not bring a very appreciable amount of material in solution. That soils are very insoluble and have a low rate of solubility has been shown by the work of Bouyoucos (2), in using the freezing-point method. That being the case, the concentration of the soil solution should be approximately inversely proportional to the moisture content of the soil. The displacement method is well adapted to such a study, for it can be used at a wide range of moisture contents. If the concentration of the soil solution obtained from a soil at different moisture contents is inversely proportional to the moisture content, it affords further proof that the method gives the true soil solution.

The relation of the moisture content and the concentration of the soil solution was studied in three soils. The soils had been in the greenhouse in a moist condition several weeks. Portions of the moist soil were weighed out; to some portions water was added to give the desired moisture content while others were allowed to dry to lower the moisture contents. Before packing in the percolators all portions except those at the higher moisture contents were passed through a coarse screen to insure thorough mixing. Displacement was started as soon after the addition of water as possible, usually within 4 to 6 hours.

Tables 4, 5 and 6 give the results obtained with Plainfield sand, Miami silt loam and Carrington silt loam. If the concentration is inversely propor-

tional to the moisture content, the freezing-point depression of the solution multiplied by the moisture content of the soil will give a constant ($D{:}M = K$). Also the parts per million of total salts in the dry soil will be a constant.

TABLE 4

The freezing-point depression of the soil solution and the total salts in Plainfield sand at varying moisture contents

MOISTURE CONTENT	FREEZING-POINT DEPRESSION OF SOLUTION	K (D.M=K)	TOTAL SALTS IN SOIL	
			Before ignition	After ignition
per cent	°C.		*p. p. m.*	*p. p. m.*
4.25	0.045	0.191	62	13.1
6.31	0.030	0.189	45	13.7
8.30	0.022	0.182	57	12.9
10.70	0.018	0.192	50	14.7
12.40	0.014	0.173	49	14.1
15.00	0.013	0.195	54	15.1

TABLE 5

The freezing-point depression of the soil solution and the total salts in Miami silt loam at varying moisture contents

MOISTURE CONTENT	FREEZING-POINT DEPRESSION OF SOLUTION	K (D.M=K)	TOTAL SALTS IN SOIL	
			Before ignition	After ignition
per cent	°C.		*p. p. m.*	*p. p. m.*
10.30	0.039	0.401	116.8	44.4
13.55	0.030	0.406	116.1	47.1
17.25	0.022	0.379–	104.3	43.4
20.62	0.018	0.371	108.9	44.8
29.41	0.013	0.382		
34.05	0.012	0.408		

TABLE 6

The freezing-point depression of the soil solution and the total salts in Carrington silt loam at varying moisture contents

MOISTURE CONTENT	FREEZING-POINT DEPRESSION OF SOLUTION	K (M.D=K)	TOTAL SALTS IN SOIL	
			Before ignition	After ignition
per cent	°C.		*p. p. m.*	*p. p. m.*
8.77	0.100	0.877	275	94.0
11.80	0.071	0.837	253	92.3
13.95	0.067	0.934	253	94.2
16.00	0.045	0.720	252	95.3
18.55	0.043	0.797	253	96.0

The results show that within experimental error K and the parts per million of total salts are constants. Assuming that the concentration of the soil solution is inversely proportional to the moisture content, as is undoubt-

edly very nearly the case, these results indicate that the true soil solution is obtained. Ischerekov (15) preformed a similar experiment and obtained results of the same order.

A comparision of results obtained by displacement and water extraction

In a study of any method it is desirable to compare results obtained by its use with those obtained by other methods. The water extraction method is the one most generally used in studying the soluble salt content of soils. It was therefore used as a means of further studying the results obtained by the displacement method. The two methods can not be expected to give the same results in all cases but the result should be of the same general order.

All nitrates are readily soluble and undoubtedly a very nearly correct quantitative determination of the nitrate nitrogen in the soil solution is

TABLE 7

Nitrate nitrogen in the dry soil as determined by the water-extraction and displacement methods

SOIL NUMBER	NITRATE NITROGEN IN THE DRY SOIL	
	Displacement method	Water extraction
	p. p. m.	*p. p. m.*
1	4.5	4.0
2	54.6	52.0
3	31.0	34.0
4	103.3	100.0
5	53.2	50.0
6	63.3	60.0
7	17.3	20.0
8	38.0	36.0
9	2.4	2.8
10	6.9	6.4

secured by the usual 1.5 water extraction. Since all of the nitrates are probably in the soil solution before the addition of water, it should be possible to obtain the same results for nitrates in the soil by using the two methods. The results obtained with the two methods on a number of soils from different field plots and the greenhouse are given in table 7.

The two methods, within experimental error, give the same result for nitrate nitrogen in the soils. We may therefore conclude that the displaced solution is of the same nitrate concentration as the solution in the soil. It is also evident that the solution is of the same concentration as the soil solution remaining in the soil. If this were not the case the results for nitrates would not agree. Probably the only difference between the displaced solution and the same solution as it existed in the soil, is that when in the soil it was under the influence of a physical force, adhesion, which held it to the soil particles.

A further comparison of the two methods was made by using Miami and Carrington silt loam and determining the total salts and calcium. The water-

extraction method cannot be expected to give exactly the same results for total salts and calcium as the displacement method, since the addition of a very large amount of water undoubtedly affects to some extent the amount of material in solution. As several investigators (17, 24) have shown, the results obtained by water extraction depend largely upon the ratio of soil to water. Therefore, it seemed desirable to use varying ratios of soil to water in the present case. The results are given in tables 8 and 9.

In both soils the displacement method gave higher results for total salts than either the 1:1 or 1:2 water extraction. Evidently the addition of these

TABLE 8

Total salts and calcium in Miami silt loam obtained by water extraction and displacement

METHOD USED	AMOUNT IN THE DRY SOIL		
	Total salts before ignition	Total salts after ignition	Calcium
	p. p. m.	*p. p. m.*	*p. p. m.*
Displacement	261	100	35.6
1:1 extraction	211	83	27.1
1:2 extraction	234	94	30.4
1:5 extraction	311	109	38.6
1:10 extraction	377	145	60.8

TABLE 9

Total salts and calcium in Carrington silt loam as determined by water extraction and displacement

METHOD USED	AMOUNT IN THE DRY SOIL		
	Total salts before ignition	Total salts after ignition	Calcium
	p. p. m.	*p. p. m.*	*p. p. m.*
Displacement	551	209	53.9
1:1 extraction	450	173	63.4
1:2 extraction	470	199	64.2
1:5 extraction	582	238	73.1
1:10 extraction	646	277	78.5

amounts of water to the soils caused a greater removal of soluble material from solution than was brought into solution by the solvent action of the water. This removal of material from solution is probably caused largely by precipitation due to the change in the nature of the solvent. When larger quantities of water were used the solvent action was greater than the precipitating effect. The point of balance of these factors will probably vary in different soils. It is probable that on some soils a 1:1 water extraction would give higher results than the displacement method. In the two soils studied the 1:5 water extraction gave approximately the same result for total salts as the displacement method.

More calcium was obtained from Miami silt loam by displacement than by a 1:1 or 1:2 water extraction, but this relation does not hold in the case of the Carrington silt loam. This difference is probably due to the calcium being present in different forms in the two soils. The Miami silt loam used was only very slightly acid, while the Carrington silt loam was strongly acid. Some results obtained with phosphorus on these two soils indicate that the phosphorus content of the soil solution is much lower than would be indicated by the usual water extraction.

The results indicate that the displacement method gives the true soil solution. Further studies of this character should give considerable information regarding the value of the water-extraction method for determining the

TABLE 10

Total salts and nitrate nitrogen in different soils obtained by the water-extraction and displacement methods

KIND OF SOIL	NO_3 NITROGEN		TOTAL SALTS IN THE DRY SOIL			
			Before ignition		After ignition	
	Displacement	Extraction	Displacement	Extraction	Displacement	Extraction
	p. p. m.	*p. p. m.*	*p. p. m.*	*p. p. m.*	*p. p. m.*	*p. p. m.*
Acid peat	791.0	840.0	7,484	11,374	2,530	3,965
Neutral peat	745.0	690.0	8,940	7,497	3,233	2,618
Clay loam	75.2	71.5	747	796	252	281
Superior clay	24.7	29.4	301	370	87	121
Plainfield sand	22.4	18.7	275	205	75	60
Hancock sand	61.2	57.0	1,512	1,400	348	325
Gray silt loam	9.7	10.8	161	223	57	124
Miami silt loam	71.0	79.8	648	732	222	256
Carrington silt loam	54.5	48.3	512	506	173	168
Waukesha silt loam	30.4	30.4	340	462	119	186

soluble material in a soil. Before final conclusions can be made regarding the value of the water-extraction method as compared with displacement further investigations will be necessary. These should include determinations of the phosphorus, potassium, calcium and magnesium in a large number of soils by the two methods.

A further comparison of the two methods was made on ten soils by using the 1:5 water extraction on all except the peats. A 1:10 extract was made with the acid peat and a 1:7.5 extract with the neutral peat. The results are shown in table 10 and confirm the conclusions drawn from the preceding data. With some soils the displacement method gave higher results for total salts, while with other soils the water extraction gave the higher results. The results for nitrate nitrogen are the same, within experimental error.

Discussion of the displacement method

The results obtained seem to prove that the displacement method gives the true soil solution. It can be used on all soil classes and at a wide range of moisture contents. By using a long soil column small amounts of the soil solution have been obtained from Carrington silt loam at a moisture content of only 6 per cent. The moisture content at which heavy soils can be most conveniently worked is slightly below the optimum for plant growth.

The method has several distinct advantages over other methods which are being used. One of its greatest advantages over the oil pressure method (18) is its simplicity and the fact that it does not require special apparatus. Ordinary glass percolators were entirely satisfactory for most of the work. If it is desirable to reduce the amount of alcohol required the displacement may be started with 200 or 300 cc. of alcohol and after this has penetrated the soil, water may be added to complete the displacement.

The greatest advantage it has over water extracts is that it gives a more correct measure of the material in solution. In addition, the solution obtained is about twenty times as concentrated as the water extract. This greatly reduces the time required for evaporation in all determinations. Five cubic centimeters was generally sufficient for a colorimetric nitrate determination and 25 cc. for a total salt determination.

The two main disadvantages of the method are the time required for displacement and the necessity of using a larger soil sample than is required by the water-extraction method. The time factor is best controlled by experience and care in packing the soil. About five times as much soil must be used as is required for water extraction.

The method seems to deserve much greater attention than it has received in the past. By its use considerable information may be obtained regarding the concentration, composition, and reaction of the true soil solution. It may also afford information that may be of value in studying results which have been obtained by water extraction and other methods.

Results obtained with the displacement method indicate that at ordinary moisture contents the concentration of the soil solution is inversely proportional to the moisture content of the soil. This does not agree with the conclusions which Bouyoucos and McCool made from results obtained with the freezing-point method. A study of the freezing-point method was therefore made to determine the cause of this disagreement.

THE FREEZING-POINT METHOD

The freezing-point method as a means of determining the concentration of the soil solution directly in the soil was first used by Bouyoucos and McCool (5, 6). It has since been used by Hoagland (13) in studying changes in the salt content of soils due to seasons and cropping. He compared results obtained by this method with those of Stewart (24) who used the water-

extraction method. The two methods gave the same general indications regarding the changes which took place in the salt content of the soils, but the water-extraction method gave from 1.5 to 5 times as much total salts as was indicated by the freezing-point method to be actually in the soil solution.

The freezing-point method as a means of measuring the concentration of the soil solution in the soil is based upon the principle that material in solution causes a depression of the freezing point of the solvent. The assumption was made that the finely divided material of the soil does not affect the freezing-point of the soil solution.

In determinations of the freezing-point depressions of soils at varying moisture contents, Bouyoucos and McCool (6) obtained results which, contrary to what would be expected, indicate that the concentration of the soil solution is not inversely proportional to the moisture content of the soil. The lowering of the freezing point of soils was found to increase approximately in geometric progression as the moisture content decreased in arithmetic progression. This was explained by the following hypothesis:

> The hypothesis assumes that a portion of the water found in the soils is inactive and does not take part in dissolving the salts in the soil, and is removed from the field of action as far as the lowering of the freezing point is concerned. Under this assumption the increase of the freezing-point depression is a geometric progression as the percentage of water increases in an arithmetic progression is explained as follows: If a clay soil, for instance, causes 15 per cent of water to become inactive, and this clay at 39 per cent of moisture produces a lowering of the freezing point of 0.075°C. and at 22 per cent 0.987°C., then at the former moisture content there is 24 per cent of water free or available to dissolve the salts in the soil, while at the latter water content there is only 7 per cent available for the same purpose. It would be natural, therefore, that the depression of the freezing point would be many times greater at the low moisture content than at the high, than would be expected from the difference in the total moisture content, just as the experimental data really indicate.
>
> This hypothesis also assumes (and the assumption seems to have been proved) that the percentage of inactive water is greater at the low than at the high moisture content and tends to decrease from the former to the latter.

Results obtained with the displacement method indicate that all of the water in the soil acts as a solvent and that there must be another explanation of the results obtained with the freezing-point method.

The concentration of the soil solution at varying moisture contents as determined by the freezing-point and displacement methods

It has already been shown in tables 4, 5, and 6 that, as determined by the displacement method, the concentration of the soil solution is inversely proportional to the moisture content of the soil. Freezing-point determinations were made at the same time on these soils. Having determined the freezing-point depression of the soil and of the displaced solution, the value for K can be calculated for both, using the equation $M.D = K$. In each case M is the moisture content of the soil and D is the freezing-point depression of the

soil or the displaced soil solution. If the concentration of the soil solution is inversely proportional to the moisture content K should be a constant. If K is not a constant it indicates that the concentration of the soil solution is not inversely proportional to the moisture content or that there is another factor influencing the freezing-point depression. The value of K for the displaced solution and the soil should be the same if the freezing-point depression in both cases is caused entirely by the salts in the soil solution. If the values for K are not the same it indicates that there are other factors affecting the freezing-point depression of the soil. The results obtained with Miami silt loam are given in table 11. Results similar to those given were obtained with Plainfield sand and Carrington silt loam.

The data show that the two methods give an entirely different indication of the concentration of the soil solution, particularly at the lower moisture contents, if it is assumed that the depression is due entirely to material in solution At a moisture content of 10.30 per cent the freezing-point depression of the soil

TABLE 11

The freezing-point depression of Miami silt loam and the displaced soil solution at different moisture contents

MOISTURE CONTENT	FREEZING-POINT DEPRESSION OF SOIL	FREEZING-POINT DEPRESSION OF SOLUTION	K FOR SOIL	K FOR SOLUTION
per cent	°C.	°C.		
10.30	0.460	0.039	4.738	0.401
13.55	0.257	0.030	3.482	0.406
17.25	0.100	0.022	1.725	0.379
20.62	0.057	0.018	1.175	0.371
29.41	0.028	0.013	0.676	0.382
34.05	0.016	0.012	0.549	0.403

indicates a concentration almost twelve times as great as is indicated by the freezing-point depression of the displaced soil solution. At the highest moisture content the two methods give nearly the same result. There are two possible explanations for the results obtained, viz., (a) the inactive or unfree water,[2] which is not supposed to act as a solvent, may be displaced and dilute the other portion of the soil solution; (b) the soil may not cause water to become inactive as a solvent but the finely divided solid material of the soil may cause a depression of the freezing point in addition to that caused by materials in solution.

The first explanation is plausible in that it is possible to displace small amounts of the soil solution at such low moisture contents that there would probably be no free water present. However, with this explanation it would be necessary to assume that unfree water is free to move capillarily. In fact, this assumption is necessary if the displacement method gives a true aliquot

[2] In this paper the terms free and unfree or inactive water are used with the meanings attached to them by Bouyoucos and McCool (6), and by Bouyoucos (3).

of the soil solution. It does not seem probable that a portion of the water would be unable to act as a solvent and at the same time be capable of capillary movement. Therefore, the second explanation may be more nearly correct than the first, as is further indicated in the following.

It is well known that colloidal solutions have the same freezing point as pure water. However, such determinations are not comparable to determinations of the freezing-point depressions of soils in which the amount of liquid present is reduced until it is all in the capillary or film condition. In a review of the literature, the writer did not find any investigations in which a deliberate study was made of the effect of finely divided material on the freezing point when the amount of liquid was so reduced. Foote and Saxton (11, 12) however, in studying the forms of water in certain hydrogels by the dilatometer method, recognized that the hydrogels caused a depression of the freezing point of the capillary water. (They defined capillary water as that water which would not freeze at 0°C., but could be frozen at lower temperatures.) Van Bemmelen and other investigators (27) have shown that water in hydrogels has a low vapor pressure. Zsigmondy, Bachmann, and Stevenson (26) have shown that the same is true for alcohol and benzene in alcogels and benzolgels. If the vapor pressure of a liquid is lowered, its freezing point also is lowered. It therefore seemed probable that the solid material of the soil may cause a depression of the freezing point of the soil solution. The results shown in table 11 indicate that this is the case. In order to obtain more conclusive data on the question, a study was made of the effect of finely divided materials on the freezing point of water, benzene and nitrobenzene. A portion of the results are presented here, but for a more detailed discussion of the procedure and results the reader is referred to another article (20).

In order to study the effect of finely divided materials upon the freezing point of liquids it is desirable to have the solid material as free as possible of substances soluble in the liquids. The materials used fulfilled this requirement very well, as is indicated by the freezing-point depressions at the highest moisture contents.

The $Fe(OH)_3$ was prepared by precipitation with NH_4OH from a cold dilute solution of the chloride. It was washed free of chlorides, air-dried and ground to pass a 200-mesh screen. The percentage of water in the $Fe(OH)_3$ is expressed on the air-dry basis. In all other cases the percentage of liquid is expressed on the oven-dry basis.

Baker's C. P. Al_2O_3 was used. It contained some material soluble in water but nothing soluble in benzene or nitrobenzene.

The freezing-point depression of water in finely divided material

The freezing-point depression of water in $Fe(OH)_3$ and Carrington silt loam was determined at varying moisture contents. The results are shown in table 12.

The results indicate a considerable depression due to the solid material. The effect due to soluble material was probably small, especially in the case of the $Fe(OH)_3$, since this material gave a depression of only 0.004°C. at a moisture content of 100 per cent. In order to explain the results obtained with $Fe(OH)_3$ by the hypothesis that it rendered part of the water unfree, it would be necessary to assume that at a moisture content of 15 per cent 14.85 per cent of water was unfree and only 0.15 per cent of water was acting as a solvent as is indicated by the following calculations. Solving for K in the equation $M.D = K$ when M is 100 and D is 0.004°C., the value of K is 0.400. Taking this value for K and solving for M when the freezing-point depression is 2.668°C. one obtains 0.15 as the value of M. In this case M is the percentage of water which would be acting as a solvent at the moisture content at which a depression of 2.668°C. is secured. The inactive water would be obtained by difference and found to be 14.85 per cent. A similar

TABLE 12

The freezing-point depression of water in $Fe(OH)_3$ and in Carrington silt loam at varying moisture contents

Fe (OH)₃		CARRINGTON SILT LOAM	
Moisture content	Freezing-point depression	Moisture content	Freezing-point depression
per cent	°C.	*per cent*	°C.
15.0	2.668	9.0	1.622
17.5	1.651	11.5	0.585
20.0	0.393	14.0	0.315
22.5	0.177	16.5	0.213
25.0	0.086	21.5	0.113
37.5	0.016	26.5	0.062
50.0	0.009	31.5	0.030
100.0	0.004	46.5	0.021

calculation for the soil shows that at a moisture content of 9.0 per cent it would be necessary to assume that only 0.60 per cent water was free and that 8.40 per cent was unfree.

The freezing-point depression of benzene and nitro-benzene in Al_2O_3 and Carrington silt loam

The use of organic liquids in which most inorganic salts are insoluble makes it possible to eliminate entirely the depression due to soluble materials. Benzene and nitrobenzene were chosen because they are readily obtained and freeze at a convenient working temperature. The results obtained with these liquids in Carrington silt loam and aluminium oxide are given in tables 13 and 14.

The results are of the same order as was obtained with the same solid material and water. Since there is no material in solution it seems that the

only possible explanation of the results is that the solid material causes a depression of the freezing point of the liquids when they are in the film or capillary condition, but does not affect their freezing point at contents above saturation. If this is the correct explanation for the results obtained with these liquids it is undoubtedly the explanation for the results obtained with soils. It may therefore be concluded that at ordinary moisture contents the freezing-point depression of the soil solution in the soil is caused by two factors, the material in solution, and the finely divided solid material of the soil.

TABLE 13

The freezing-point depression of benzene in Carrington silt loam and aluminium oxide

CARRINGTON SILT LOAM		ALUMINIUM OXIDE	
Benzene	Freezing-point depression	Benzene	Freezing-point depression
per cent	°*C.*	*per cent*	°*C.*
5.0	0.660	30.0	1.337
7.5	0.355	35.0	0.682
10.0	0.150	40.0	0.492
12.5	0.060	45.0	0.326
15.0	0.033	50.0	0.212
20.0	0.025	55.0	0.115
25.0	0.010	65.0	0.052
37.5	0.000	75.0	0.030
		100.0	0.000

TABLE 14

The freezing-point depression of nitrobenzene in Carrington silt loam and aluminium oxide

CARRINGTON SILT LOAM		ALUMINIUM OXIDE	
Nitrobenzene	Freezing-point depression	Nitrobenzene	Freezing-point depression
per cent	°*C.*	*per cent*	°*C.*
12.5	1.630	50.0	1.720
15.0	1.200	60.0	1.175
17.5	0.780	70.0	0.810
20.0	0.510	80.0	0.580
25.0	0.230	90.0	0.340
30.0	0.130	100.0	0.200
37.5	0.075	150.0	0.020
50.0	0.000	200.0	0.000

The relation between the freezing-point depression due to solid material and that due to material in solution

In order to determine whether or not the depression due to solid material and that due to material in solution are additive in their effect on the freezing point, samples of aluminium oxide were moistened with water and other samples were moistened with a sugar solution which had a freezing-point

depression of 0.126°C. A sugar solution was used because there is no possibility of a chemical reaction between the sugar and aluminium oxide. If the two factors are additive the difference between the freezing-point depressions at any moisture content should be 0.126°C., the depression due to sugar. Adsorption may influence the results to some extent. The differences obtained are given in the last column of table 15.

These results and others obtained when sugar solutions and a $Ca(NO_3)_2$ solution were used in soils, kaolin, silica and ferric hydroxide, prove that the two factors are very nearly additive in their effect on the freezing point. The differences found at the three lower moisture contents are easily within the limit of experimental error, which is quite large at low moisture contents. The presence of sugar did not affect the general order of results.

The results afford further evidence that the great increase in the freezing-point depression at the lower moisture contents is not due to part of the water being withdrawn from the rôle of a solvent by the solid material. If the

TABLE 15

The freezing-point depression of water and a sugar solution in aluminium oxide at different moisture contents

MOISTURE CONTENT	FREEZING-POINT DEPRESSION WITH WATER	FREEZING-POINT DEPRESSION WITH SOLUTION	FREEZING-POINT DEPRESSION DUE TO THE SUGAR
per cent	°C.	°C.	°C.
25.0	2.118	2.290	0.172
30.0	1.227	1.312	0.075
35.0	0.650	0.740	0.090
40.0	0.370	0.500	0.130
50.0	0.220	0.344	0.124
75.0	0.075	0.195	0.120
100.0	0.053	0.173	0.120

aluminium oxide had rendered any of the water unfree the solution would have been greatly concentrated at the lower moisture contents and the depression due to the sugar would have been many times that shown in the table. The depression of the freezing point of water at 25 per cent was forty times as great as at 100 per cent. If this had been caused by part of the water being withdrawn from the rôle of a solvent, and the same amount had been withdrawn when the sugar solution was added, the depression of the sugar solution at 25 per cent would have been 6.920°C. instead of 2.290°C. The depression due to the sugar alone at this moisture content would have been 4.802°C. instead of only 0.172°C., the experimental value.

The freezing-point depression of soils at their moisture equivalent

The freezing-point depression of soils at medium to low moisture contents is caused in large part by the solid material. The force which holds the water on the soil particles probably causes the freezing-point depression. If a num-

ber of soils are subjected to the same centrifugal force, and come to an equilibrium with this force, they will retain different percentages of water. These different percentages of water which are retained will be held with equal forces in the different soils. Since this same force probably causes the freezing-point depression, it should be possible to reduce all soils to the same freezing-point depression, after the removal of soluble salts, by subjecting them to the same centrifugal force.

In a determination of the moisture equivalent of soils the soil is subjected to a centrifugal force of 1000 times gravity. The moisture content after centrifuging is that at which the force of attraction between the soil and water is equal to this centrifugal force. In this manner the force holding water in different soils may be brought to a uniform value. Therefore the freezing-point depression of the soils, due to solid material, should be equal at the moisture contents represented by their moisture equivalents.

TABLE 16

The freezing-point depression of washed soils at their moisture equivalent

SOIL	MOISTURE EQUIVALENT	FREEZING-POINT DEPRESSION
		°C.
Carrington silt loam	21.57	0.055
Knox silt loam	25.40	0.057
Mellen loam	16.39	0.053
Superior clay	21.84	0.061
Plainfield sand	4.73	0.076
Miami silt loam	26.47	0.052
Silty clay loam	33.29	0.061
Fine sandy loam	12.49	0.057
Peat	111.60	0.061
Kaolin	38.12	0.046
Silica	19.14	0.057
Aluminium oxide	21.56	0.043

In order to determine the freezing-point depression due to solid material it is necessary to remove the soluble material as far as possible. One-hundred-gram samples of different soils, silica, kaolin and aluminium oxide were washed with 1200 to 1500 cc. of distilled water to remove soluble salts. The samples were then dried in an electric oven at 105°C. The moisture equivalent was determined in the usual manner. After centrifuging the freezing-point determinations were made on the soils. The speed of the centrifuge was slightly less than that which gives a force of 1000 times gravity. This caused slightly high results for the moisture equivalent and hence low results for the freezing-point depression, due to solid material, at the true moisture equivalent. There were undoubtedly several sources of error, such as the incomplete removal of the soluble material, puddling in making the determination of the moisture equivalent, and evaporation in transferring the soil from the moisture cups to the freezing-point tubes. The results are shown in table 16.

The results indicate that at the moisture equivalent the depression of the freezing point due to solid material is very nearly the same in different soils and certain artificial materials. This can be explained only by the assumption that the same force which retains the moisture causes the freezing-point depression. The values are not exactly the same but are within experimental error. The low values for kaolin and aluminium oxide are probably due to the fact that these materials pack very tightly in the centrifuge preventing the water from being readily thrown out. The high result for the Plainfield sand may be due to the extreme readiness with which the water is removed from such a coarse soil.

The results indicate that the freezing-point method could be used to determine the salt content of soils at their moisture equivalent. At the moisture equivalent the depression due to solid material is probably a constant, about 0.050°C. By determining the freezing-point depression of a soil at its moisture equivalent and subtracting this constant, the freezing-point depression due to soluble material would be obtained. In using such a procedure great care should be taken that the true moisture equivalent is used in all cases, for a slight error in the moisture content would decidedly affect the freezing-point depression due to solid material. This procedure is rather long, so it is doubtful if it would be of much value. If the freezing-point method is used it would be more convenient and probably more accurate to make the determinations at moisture contents above saturation, entirely eliminating the depression caused by the solid material.

Discussion of the freezing-point method

The freezing-point method does not give a measure of the concentration of the soil solution in the soil at ordinary moisture contents, for it has been shown that finely divided material causes a depression of the freezing point of a liquid in the film or capillary condition. The force holding the liquid on the solid material and causing the freezing-point depression is adhesion. When the amount of water in the soil is increased a point is reached at which some of the soil solution is so distant that this force no longer affects its freezing point. This point is probably the point of saturation. Probably at any moisture content below saturation the solid material causes a depression of the freezing point of the soil solution. At moisture contents above saturation the depression becomes due entirely to material in solution.

The freezing-point method has been used by Bouyoucos and his associates to determine the lime requirement of soils (1), the velocity of reactions between soils and chemical reagents (4), the solubility of soils (2), and the absolute salt content of soils (7). In these investigations the moisture content was always very high, varying from 66 to several hundred per cent. At those moisture contents the depression is due entirely to materials in solution and the method gives a satisfactory measure of the soluble material present. It is well adapted to such studies and used under these conditions has given valuable results.

SUMMARY

It is desirable in studying problems of soil fertility, plant nutrition and related subjects to have a method with which the true soil solution may be obtained from a soil at ordinary moisture contents in sufficient quantities for analytical work. In a review of the literature it was found that Ischerekov (15), using the displacement method, obtained results which indicate that the method gives the true soil solution in quantities sufficient for most purposes. Hence it seemed desirable to make a study of the method and compare it with other methods which are now being used in studying the concentration and composition of the soil solution.

The method consists of packing the moist soil in a cylinder provided with an outlet at the bottom. Ethyl alcohol is then poured on top of the soil column and as it penetrates the soil it displaces some of the soil solution which forms a zone of saturation below the alcohol. This zone increases in depth as it is continually forced downward by the alcohol. When the saturated zone reaches the bottom of the soil column the clear soil solution, free of alcohol, drops from the soil as gravitational water.

A study was made of the effect of different displacing liquids on the time required for displacement, the percentage of the soil solution displaced and the composition of the displaced solution. The concentration of successive portions of the displaced soil solution was determined and also the concentration of the soil solution obtained from the soils at different moisture contents. The amount of total salts, nitrates, and calcium obtained from the soil by displacement was compared with the amount secured from the soil by water extraction.

Results obtained with the displacement method did not agree with the conclusions of Bouyoucos and McCool (6), drawn from a study of the freezing-point depressions of soils, with regard to the concentration of the soil solution at different moisture contents and the forms of water in the soil. Therefore a study was made of the freezing-point method and factors affecting the freezing-point depression of the soil solution in the soil. The effect of finely divided material on the freezing point of water, benzene and nitrobenzene was studied. A summary of the results and conclusions is given below.

(a) Ethyl alcohol was found to be more satisfactory as a displacing liquid than water, methyl alcohol, acetone, or liquids non-miscible with water.

(b) The composition of the soil solution obtained by displacement was not influenced by the displacing liquid used.

(c) Successive portions of the displaced solution gave the same freezing-point depression and contained the same amount of total salts.

(d) The concentration of the displaced solution was found to be inversely proportional to the moisture content of the soil.

(e) The displacement method gave the same amount of nitrate nitrogen and approximately the same amount of total salts as a 1:5 water extraction of the soil.

(f) The method seems to be well adapted to a study of the composition and reaction of the soil solution under any condition.

(g) Finely divided material was found to cause a depression of the freezing point of water, benzene, and nitrobenzene when the amount of liquid was reduced until it was in the film or capillary condition.

(h) The freezing-point method does not give a measure of the concentration of the soil solution directly in the soil at ordinary moisture contents of the soil.

(i) At high moisture contents, probably only above saturation, the freezing-point method gives a measure of the concentration of the soil solution.

(j) The freezing-point depression due to solid material at the moisture equivalent was found to be very nearly a constant for a number of soils.

(k) A soil does not cause a considerable amount of water to be removed from the role of a solvent, as has sometimes been assumed.

A subsequent article will be devoted to the classification of the soil moisture

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THE EFFECT OF STRAW ON THE BIOLOGICAL SOIL PROCESSES[1]

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INTRODUCTION

In the wheat country of Eastern Washington, the addition of straw is practically the only method, under the present practices of farming, of returning humus to the soil. It is a common observation that the addition of wheat straw to the soil decreases the following crop yield. The practice of burning wheat stubble on the farm is common, but such procedure is detrimental to permanent agriculture. It is therefore important to determine why the addition of straw to the soil does cause a decrease in the following crop yield and whether any practice will overcome the loss. It was with this purpose in view that the work was taken up.

HISTORICAL

The following discussion refers to the work that has been done on the effect of straw and allied materials upon soils under various conditions.

Störmer (10) showed that in a series of pot experiments on heavy and light soils, the addition of straw to green manure reduced the efficiency of the nitrogen in the green manure. The second year the results were better. Straw alone reduced the yield—the finer the straw the smaller the yield. The addition of carbon bisulfide to the soil to which straw had been added doubled the yield.

Von Seelhorst and Freckmann (13) worked on the effect of aeration and moisture upon the addition of straw and strawy manures on loam and sandy soils in pots. Their work showed that when nitrates were applied denitrification was proportional to the applications of straw. In the case of the loam, the application of straw alone was more injurious.

Von Seelhorst and Freckmann (14) showed that the addition of lime and sulfuric acid lessens the injurious effect of straw on the yield, but does not overcome it.

Stutzer (11) carried on pot experiments with buckwheat followed by mustard to determine whether the injurious effects caused by the addition

[1] Contribution from the Washington Agricultural Experiment Station, State College of Washington, Pullman, Washington.

of straw and similar organic substances could be overcome by the addition of ammonium sulfate. He found the growth poorer with ammonium sulfate than with sodium nitrate. The addition of calcium carbonate increased the injurious effect of the ammonium sulfate.

Chirikov and Shmuk (3) experimented on the influence of moisture and straw upon denitrification in sandy loam. The work was carried out in pots. At a constant moisture the yield of oats decreased as the straw was increased, and as the moisture decreased the yield decreased. The addition of calcium carbonate with the straw decreased the injurious effect of the straw but did not overcome it. The authors hold that the diminished yield is not due to denitrification in the strict sense, but that nitrates are converted into insoluble nitrogen compounds which are less assimilable by the plants.

Bischoff (1) conducted pot experiments at Göttingen, in which he turned under straw, 10 weeks, 4 weeks, and immediately before planting. He grew mustard and buckwheat on a sandy and a clayey soil. He found that on the sandy soil the use of straw was practically always followed by a smaller yield of dry matter and of nitrogen. The injurious effect was less in the presence of sodium nitrate. On clayey soils the straw did not uniformly decrease the yield. With sodium nitrate the early application of straw produced the greatest depression in yield. In the absence of sodium nitrate the late application of straw caused the greatest loss.

Löhnis and Green (7) state that "straw humus interfered with the process of nitrification in the same manner as the undecomposed straw."

Von May (12), worked on the effect of straw fertilizers on potatoes. He states "the results show that the availability of organically combined nitrogen was depressed by the presence of a nitrogen-free organic substance," as in the case of straw. He suggests that the loss in available nitrogen is due to the appropriation of the soluble nitrogen by microorganisms which use the nitrogen-free substances as sources of energy.

Doryland (4) reports, as a result of his tests:

> Oats grown in pots containing quartz sand are greatly reduced in yield when dextrose is added in the presence of limited amounts of plant food. When dextrose is added in the presence of abundant plant food the reduction in oat yield is less. Soils have a definite ammonia and nitrate consuming power. The addition of dextrose or straw temporarily increases it. When large quantities of straw (20 tons to the acre) are added to soil, there is a marked decrease in the ammonifying power and a marked increase in the ammonia and nitrate-consuming power. Plowing under crop residues affects the soil in a similar way, but to a less extent. The disturbing influence of organic residue is largely due to the excess energy material with a marked temporary increase in multiplication of microorganisms, and a proportional assimilation of plant food elements followed upon exhaustion of excess energy material, by an increased liberation of plant food. The terms "beneficial" and "detrimental" bacteria when applied to saprophytic organisms have only a relative value. Under a certain proportion of energy to nutrients, the "beneficial" organism may become "detrimental," and vice versa.

DISCUSSION

There are several possible explanations of what takes place in the soil after the addition of wheat straw. The addition of cellulose to the soil in the form of wheat straw may have a toxic effect on the nitrate-forming bacteria and thus inhibit the formation of nitrates. It may be that the cellulose-fermenting organisms use up the nitrates as a food for themselves and thus lower the nitrogen supply of the plants. Another point that suggests itself is that the straw acts as a source of energy to a host of soil bacteria, which then use the nitrates as their source of nitrogen, build up organic nitrogen, probably throw off nitrogen in the form of organic nitrogenous matter as a waste product, thus deprive the plants of their soluble nitrogenous food.

OUTLINE OF WORK

The direct object of the work in hand was to find the effect of adding straw to the soil on the biological agents of the soil, to study the effect of straw on different groups of bacteria and their activities, and as a result of these investigations to devise, if possible, some method of adding humus to the soil in the form of wheat straw, without subsequent ill effects. Specifically, the following work was undertaken to study: first, the effect of adding varying amounts of straw to the soil upon nitrate production and upon the total nitrogen content of the soil, to find out whether nitrogen is really lost or merely transformed in the soil; second, the effect of straw upon the number and the type of bacteria in the soil; third, the effect of straw upon nitrogen fixation, ammonification and denitrification; fourth, the isolation of nitrate-forming bacteria and the effect of straw and pure cellulose upon them.

EXPERIMENTAL

The effect of different amounts of straw on nitrate formation and the total nitrogen content of the soil[2]

To 100 gm. of sieved soil[3] contained in glass tumblers, 0.1, 0.2, 0.3, 0.4, 05, 0.6, 0.7, 0.8, 0.9, 1.0, 2.0, 3.0, 4.0 and 5.0 per cent of finely chopped wheat straw was added. The moisture content was adjusted to 19 per cent, and 0.2 gm. of ammonium sulfate was added to each tumbler as well as to a check which received no straw. The work was carried out in duplicate. The tumblers were covered and weighed and incubated at room temperature, about 21°C. The moisture content was adjusted weekly. Determinations of the nitrate content by the zinc-iron reduction method (6), and the total nitrogen by the modified Gunning method to include nitrates, were made immediately, and at the end of 6, 12, 18 and 24 weeks.

[2] Credit is due Mr. Milton Brandt for most of the analyses in this section.

[3] All soil used was Palouse field soil.

From an analysis of tables 1, 2 and 3, it is apparent that the addition of wheat straw has an effect on the formation of nitrates in the soil under laboratory conditions. After the 6 and 12-week periods, straw between the

TABLE 1

Nitrate nitrogen per 100 gm. of soil—Average of two determinations

AMOUNT OF STRAW ADDED	AT BEGINNING	END OF 6 WEEKS	END OF 12 WEEKS	END OF 18 WEEKS	END OF 24 WEEKS
per cent	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
0.1	9.72	12.25	22.84	27.58	23.52
0.2	9.72	14.81	22.82	32.76	25.56
0.3	9.72	15.12	21.84	34.10	23.38
0.4	9.72	14.91	19.88	27.16	25.38
0.5	9.72	13.86	21.28	31.78	25.76
0.6	9.72	16.36	21.70	31.78	23.10
0.7	9.72	13.16	22.68	29.26	21.60
0.8	9.72	12.04	19.60	27.02	22.12
0.9	9.72	12.18	20.66	27.86	22.02
1.0	9.72	13.72	15.54	32.28	22.54
2.0	9.72	11.06	13.58	27.02	18.48
3.0	9.72	12.42	14.00	19.28	16.52
4.0	9.72	9.26	8.68	18.62	14.56
5.0	9.72	7.84	9.52	13.36	7.56
Check	9.72	13.44	19.74	34.72	26.74

TABLE 2

Increase or decrease in nitrate nitrogen over the nitrate nitrogen in the beginning

AMOUNT OF STRAW ADDED	END OF 6 WEEKS	END OF 12 WEEKS	END OF 18 WEEKS	END OF 24 WEEKS
per cent	*mgm.*	*mgm.*	*mgm.*	*mgm.*
0.1	2.53	13.12	17.86	13.80
0.2	5.09	13.10	23.04	15.84
0.3	5.40	12.12	24.38	13.66
0.4	5.19	10.16	17.44	15.66
0.5	4.14	11.56	22.06	16.04
0.6	6.64	11.98	22.06	13.38
0.7	3.44	13.92	19.54	11.88
0.8	2.32	9.88	17.30	12.40
0.9	2.46	12.44	16.14	12.30
1.0	4.00	5.82	22.56	12.82
2.0	1.32	3.86	17.30	8.76
3.0	2.70	4.28	9.56	6.80
4.0	−0.46	0.96	8.90	4.84
5.0	−1.88	−0.20	3.64	−1.16
Check	3.72	10.02	25.00	17.02

amounts of 0.1 per cent and 0.7 per cent actually stimulated the formation of nitrates over that in the check to which no straw had been added. After the 18 and 24-week periods, in every case there was a loss in nitrate formation over the check.

Table 4 shows that if straw inhibits the formation of nitrates or actually causes a decrease in the original nitrate content, as is the case with the 4 and 5 per cent straw, there is no loss in total nitrogen in the soil. The nitrates

TABLE 3

Increase or decrease in nitrate nitrogen over the increase in the check for each period

AMOUNT OF STRAW ADDED	END OF 6 WEEKS	END OF 12 WEEKS	END OF 18 WEEKS	END OF 24 WEEKS
per cent	*mgm.*	*mgm.*	*mgm.*	*mgm.*
0.1	−1.29	3.10	−7.14	−3.22
0.2	1.37	3.08	−1.96	−1.18
0.3	1.68	2.10	−0.62	−3.36
0.4	1.47	0.14	−7.56	−1.36
0.5	0.42	0.54	−2.94	−0.98
0.6	1.70	0.96	−2.94	−3.64
0.7	0.72	2.94	−5.46	−5.14
0.8	−1.40	−0.14	−7.70	−4.62
0.9	−1.36	0.42	−6.86	−4.72
1.0	0.28	−4.30	−2.44	−4.20
2.0	−2.38	−6.39	−7.70	−8.26
3.0	−1.02	−5.94	−15.44	−10.22
4.0	−4.28	−9.06	−16.10	−12.18
5.0	−5.86	−10.22	−21.36	−19.18

TABLE 4

Total nitrogen per 10 gm. of soil—Average of two determinations

AMOUNT OF STRAW ADDED	AT THE BEGINNING	END OF 6 WEEKS	END OF 12 WEEKS	END OF 18 WEEKS	END OF 24 WEEKS
per cent	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
0.1	17.35	19.25	23.03	26.95	20.83
0.2	17.71	19.67	23.98	28.28	24.01
0.3	17.29	20.58	23.13	25.20	20.02
0.4	17.71	19.76	21.77	30.66	19.57
0.5	19.18	20.93	28.42	28.07	20.08
0.6	17.78	19.81	26.45	27.02	19.39
0.7	18.06	18.48	23.80	25.55	19.71
0.8	18.34	18.69	26.04	23.03	19.11
0.9	19.32	20.02	23.66	23.80	20.02
1.0	19.28	18.96	23.80	25.99	21.19
2.0	18.34	20.51	23.45	26.04	20.55
3.0	18.06	22.33	24.12	27.09	21.16
4.0	18.11	21.49	25.13	24.22	21.16
5.0	18.90	20.65	21.63	25.84	20.81
Check	18.20	19.53	21.98	25.55	19.97

are probably transformed to organic nitrogenous matter, and there is no loss in total nitrogen due to the straw. The amounts of nitrogen vary somewhat but the amounts at the end of each period do not lie very far apart. The addition of straw in no way interferes with the normal increase in total nitrogen.

In order to check up on this work and to find out what happens after the 24-week period, the work was repeated covering a 48-week period. The analyses were made for nitrates and total nitrogen every 6 weeks.

TABLE 5

Nitrate nitrogen per 100 gm. of soil—Average of two determinations

AMOUNT OF STRAW ADDED	AT BEGINNING	END OF 6 WEEKS	END OF 12 WEEKS	END OF 18 WEEKS	END OF 24 WEEKS	END OF 30 WEEKS	END OF 36 WEEKS	END OF 40 WEEKS	END OF 48 WEEKS
per cent	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
0.1	10.25	12.16	17.50	19.18	19.32	30.46	25.48	26.04	26.90
0.2	10.25	11.26	17.28	19.32	23.96	27.02	21.98	25.20	27.44
0.3	10.25	10.69	17.50	17.44	25.94	21.56	24.12	25.32	24.92
0.4	10.25	12.13	17.22	17.55	19.78	21.66	23.60	23.52	24.22
0.5	10.25	13.16	18.62	15.92	21.00	24.22	27.30	26.60	24.92
0.6	10.25	11.62	21.84	22.68	26.80	23.47	22.54	23.80	23.80
0.7	10.25	12.60	16.19	12.32	22.60	22.96	22.02	21.00	25.06
0.8	10.25	10.27	13.44	14.65	16.80	17.78	20.02	23.66	18.48
0.9	10.25	10.50	13.58	12.88	17.64	24.92	21.28	23.80	21.56
1.0	10.25	9.80	15.12	24.64	18.76	19.32	18.48	19.04	17.92
2.0	10.25	9.05	12.55	18.90	17.50	13.86	21.72	19.18	17.08
3.0	10.25	9.24	17.36	19.04	14.30	14.98	17.78	17.78	17.08
4.0	10.25	9.52	13.72	17.36	6.86	11.06	9.38	9.38	9.97
5.0	10.25	10.22	13.30	18.64	7.84	7.84	9.80	10.50	10.50
Check	10.25	11.20	14.70	14.28	17.74	17.50	24.36	24.36	24.50

TABLE 6

Increase or decrease in nitrate nitrogen over the nitrate nitrogen at the beginning

AMOUNT OF STRAW ADDED	END OF 6 WEEKS	END OF 12 WEEKS	END OF 18 WEEKS	END OF 24 WEEKS	END OF 30 WEEKS	END OF 36 WEEKS	END OF 40 WEEKS	END OF 48 WEEKS
per cent	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
0.1	1.91	7.25	8.93	9.07	20.21	15.23	15.79	16.65
0.2	1.01	7.03	9.07	13.71	16.77	11.73	14.95	17.19
0.3	0.24	7.25	9.17	15.69	11.31	13.87	15.07	14.67
0.4	1.88	6.72	7.30	9.52	11.41	13.35	13.27	13.97
0.5	2.91	8.37	5.67	10.74	13.97	17.05	16.35	14.67
0.6	1.37	11.59	12.43	16.55	13.22	12.29	13.55	13.55
0.7	2.35	5.94	2.07	12.35	12.71	11.77	10.75	14.81
0.8	0.02	3.19	4.40	6.55	7.53	9.77	13.41	8.23
0.9	0.25	3.33	2.63	7.39	14.67	11.03	13.55	11.21
1.0	−0.45	4.87	4.39	8.51	9.07	8.23	8.79	7.67
2.0	−1.20	2.30	8.65	7.25	3.61	11.47	9.93	7.73
3.0	−1.01	7.11	8.79	4.05	4.73	7.53	7.53	7.73
4.0	−0.73	3.47	7.11	−3.39	0.81	−1.87	−0.87	−0.28
5.0	−0.03	3.05	8.39	−2.41	−2.41	−0.45	0.25	0.25
Check	0.95	4.45	4.03	7.51	7.49	14.11	14.11	14.25

The data shown in tables 5, 6 and 7 are not as striking as the results obtained previously over the 24-week period. After the 30-week period, however, the results ran very much the same as in the 18 and 24-week periods of the

former set. The loss in nitrate formation after the 30-week period seems to increase with the increase in the straw. This is especially noticeable in the cases where 4 and 5 per cent straw applications were made. It is again evident that the straw has a marked influence on nitrate formation.

TABLE 7

Increase or decrease in nitrate nitrogen over the increase in the check for each period

AMOUNT OF STRAW	END OF 6 WEEKS	END OF 12 WEEKS	END OF 18 WEEKS	END OF 24 WEEKS	END OF 30 WEEKS	END OF 36 WEEKS	END OF 40 WEEKS	END OF 48 WEEKS
per cent	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
0.1	0.96	2.80	4.90	1.46	12.72	1.12	1.68	2.40
0.2	0.06	2.58	5.04	6.20	9.28	−2.38	0.84	2.94
0.3	−0.71	2.80	5.14	8.18	3.82	−0.24	0.96	0.42
0.4	0.93	2.27	3.27	2.01	3.92	−0.76	−0.84	−0.28
0.5	1.96	3.92	1.64	3.23	6.48	2.84	2.24	0.42
0.6	0.42	7.04	8.40	9.04	5.73	−1.82	−0.56	−0.70
0.7	1.40	1.49	−1.96	4.84	5.22	−2.34	−3.36	0.56
0.8	−0.93	−0.26	0.37	−0.96	0.04	−4.34	−0.70	−6.02
0.9	−0.70	−1.12	−1.40	−0.12	7.18	−2.08	−0.56	−3.04
1.0	−0.50	0.42	0.36	1.00	1.58	−5.88	−5.32	−6.58
2.0	−2.15	−2.15	4.63	−0.26	−3.88	−2.64	−4.18	−6.52
3.0	−1.96	2.76	4.76	−3.46	−2.76	−6.58	−6.58	−6.52
4.0	−1.68	0.02	3.08	−10.90	−6.68	−15.98	−14.98	−14.53
5.0	−0.98	−1.40	4.36	−9.92	−9.90	−14.66	−13.86	−14.00
Check								

TABLE 8

*Total nitrogen per 10 gm. of soil—Average of two determinations**

AMOUNT OF STRAW	AT BEGINNING	END OF 6 WEEKS	END OF 12 WEEKS	END OF 18 WEEKS	END OF 24 WEEKS	END OF 30 WEEKS	END OF 36 WEEKS	END OF 48 WEEKS
per cent	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
0.1	19.72	21.62	21.91	22.33	23.17	20.44	23.24	18.48
0.2	18.55	21.42	22.19	22.47	24.13	20.48	20.86	19.39
0.3	18.51	19.74	21.56	22.54	24.28	20.33	21.14	20.02
0.4	18.49	21.70	21.77	23.45	24.20	20.79	22.96	20.93
0.5	18.13	19.39	20.23	23.45	24.50	21.00	22.26	22.26
0.6	18.20	20.51	20.44	23.17	22.89	20.44	22.82	22.64
0.7	18.13	21.67	22.75	22.08	23.83	19.81	22.26	22.47
0.8	18.27	23.03	22.96	21.63	23.66	18.20	22.12	22.26
0.9	19.23	23.03	23.10	21.63	24.22	18.20	21.84	21.98
1.0	18.55	21.62	23.45	22.05	23.71	20.48	21.70	24.99
2.0	19.25	21.96	23.24	22.68	23.80	21.07	21.98	22.26
3.0	19.69	22.89	21.98	22.72	24.36	20.37	22.84	24.64
4.0	21.07	21.46	22.05	22.39	24.08	20.30	22.84	25.41
5.0	20.76	22.19	22.68	22.61	23.52	21.84	22.54	21.49
Check	18.62	21.98	20.93	22.12	22.75	21.84	21.41	20.09

* On account of an accident no determinations were made at the end of 40 weeks.

An examination of table 8 shows results that closely approximate those obtained previously. No nitrogen is lost, so the nitrates are only changed to some other form of nitrogen.

Straw in large amounts causes a decrease in the formation of nitrates. The process of nitrification is probably not interfered with (it will be shown later that the cellulose of straw has no harmful effect on nitrate bacteria), but as fast as the nitrates are formed they are used by bacteria that obtain carbon from the straw. (That the number of bacteria increase with an increase in the amount of straw is shown in a subsequent portion of this paper.)

TABLE 9

Nitrate nitrogen per 100 gm. of soil—Average of two determinations

AMOUNT OF STRAW	AT BEGINNING	END OF 6 WEEKS	END OF 12 WEEKS	END OF 18 WEEKS
per cent	*mgm.*	*mgm.*	*mgm.*	*mgm.*
0.1	52.93	47.88	50.96	52.76
0.2	52.93	48.58	51.24	52.62
0.3	52.93	49.66	52.71	51.92
0.4	52.93	49.28	51.38	50.80
0.5	52.93	49.42	51.38	53.34
0.6	52.93	49.14	47.88	53.20
0.7	52.93	42.98	50.12	52.04
0.8	52.93	40.70	48.72	53.76
0.9	52.93	41.58	47.18	52.36
1.0	52.93	43.54	46.06	49.00
2.0	52.93	37.94	46.43	46.48
3.0	52.93	37.66	45.78	44.38
4.0	52.93	29.26	46.06	43.40
5.0	52.93	30.24	47.18	42.84
Check	52.93	41.30	52.30	53.90

The effect of straw on nitrates already in the soil

In the foregoing study of the effect of straw on nitrification, the tests were made on the influence of straw in transforming an ammonium compound to nitrates. The question arises, what happens to nitrates already in the soil when straw is added? With this problem in view, 50 mgm. of nitrate nitrogen in the form of sodium nitrate was added to 100 gm. of soil, to which had been added the various percentages of straw. Nitrate analyses were made immediately and at the end of 6, 12 and 18 weeks. The results of the analyses are given in the tables 9, 10 and 11.

At the end of 6 weeks there was a loss in nitrates in all cases. There was less loss between 0.1 and 0.7 per cent straw than in the check. Above 1 per cent straw there is a very much greater loss than in the check.

There was a loss in nitrates in all cases at the end of the 12-week period. The loss in the case of the check was very small. In all except one there was a greater loss when straw had been added. In general, as the straw increased the loss increased.

At the end of the 24-week period there was a loss in nitrates in every case but in the check. The loss is greatest above 1 per cent straw.

TABLE 10

Increase or decrease in nitrate nitrogen over the nitrate nitrogen in the beginning

AMOUNT OF STRAW ADDED	END OF 6 WEEKS	END OF 18 WEEKS	END OF 18 WEEKS
per cent	*mgm.*	*mgm.*	*mgm.*
0.1	−5.05	−1.97	−0.17
0.2	−4.35	−1.69	−0.31
0.3	−3.27	−0.22	−1.01
0.4	−3.65	−1.55	−2.13
0.5	−3.51	−1.55	0.41
0.6	−3.79	−5.05	0.27
0.7	−9.95	−2.81	−0.89
0.8	−12.23	−4.21	0.83
0.9	−10.35	−5.75	−0.57
1.0	−8.39	−6.87	−3.93
2.0	−14.99	−6.50	−6.45
3.0	−15.27	−7.15	−8.55
4.0	−23.67	−6.87	−9.53
5.0	−22.69	−5.75	−10.09
Check	−11.63	−0.59	1.03

TABLE 11

Increase or decrease over the increase or decrease in nitrate nitrogen in the check at the different periods

AMOUNT OF STRAW ADDED	END OF 6 WEEKS	END OF 12 WEEKS	END OF 18 WEEKS
per cent	*mgm.*	*mgm.*	*mgm.*
0.1	6.58	−1.38	−1.20
0.2	7.28	−1.10	−1.34
0.3	8.36	0.37	−2.04
0.4	7.98	−0.96	−3.47
0.5	8.12	−0.96	−0.62
0.6	7.84	−4.46	−0.76
0.7	1.68	−2.22	−1.92
0.8	−1.60	−3.62	−0.20
0.9	1.28	−5.16	−1.60
1.0	2.24	−6.28	−4.96
2.0	−3.36	−5.91	−7.48
3.0	−3.64	−6.56	−9.58
4.0	−12.06	−6.28	−10.56
5.0	−11.06	−5.16	−11.12
Check			

It is evident that straw has an effect on nitrates present in the soil and as the amount of straw increases the harmful effect increases.

The effect of straw on ammonification

The various amounts of straw were added to 100 gm. of soil in tumblers. After the moisture had been adjusted 1 per cent casein was added to one set. The other set stood a week and then 1 per cent casein was added to it. After the addition of the casein the soil was incubated one week. Magnesium oxide and water were added to the 100 gm. of soil and the ammonia determined by distillation.

Practically all the bacteria in the soil are ammonifiers. Straw, as is shown in another part of the paper, stimulates bacterial reproduction. It was thought that if the soil stood some time with the straw, greater ammonification would take place as a result of the increase in the number of bacteria. It was with

TABLE 12

Ammonia per 100 gm. of soil—Average of two determinations

AMOUNT OF STRAW	A. CASEIN APPLIED AT ONCE	B. CASEIN APPLIED 1 WEEK AFTER STRAW
per cent	*mgm.*	*mgm.*
0.1	128.27	129.55
0.2	126.14	124.10
0.3	122.82	132.34
0.4	125.89	118.49
0.5	124.10	126.48
0.6	113.24	126.91
0.7	126.99	127.50
0.8	117.81	125.29
0.9	117.97	126.65
1.0	110.16	111.27
2.0	98.52	89.41
3.0	81.36	98.10
4.0	79.31	94.95
5.0	70.13	68.68
Check—no straw	132.17	125.38
Check—no casein, no straw	5.44	3.32

this point in mind that the soil plus the various amounts of straw was allowed to stand a week before the addition of the casein. In table 12, column A, the ammonia formed in one week is recorded. Column B shows the ammonia formed in one week where the casein was applied one week after the addition of the straw.

It is evident that above 0.9 per cent straw a harmful effect is exerted upon the process of ammonification. As the straw increases the ammonia decreases. This is true in both cases. The figures in column B do not show the increased ammonification due to the increase in bacterial numbers. It is likely that as much ammonia is formed above 0.9 per cent straw, but since there are enormous numbers of bacteria using the straw as a source of carbon, they may use the ammonia as a source of nitrogen.

The effect of straw on the fixation of atmospheric nitrogen

In order to complete the study of the biological activities in which soil bacteria function, the writer next took up the effect of straw on the fixation

TABLE 13

The effect of straw on nitrogen fixation; nitrogen 10 gm. of soil—Average of two determinations

AMOUNT OF STRAW ADDED	AT BEGINNING	END OF 3 WEEKS	END OF 6 WEEKS
per cent	*mgm.*	*mgm.*	*mgm.*
0.1	10.01	12.04	13.72
0.2	9.97	12.11	12.81
0.3	9.78	12.74	13.16
0.4	10.15	12.25	12.46
0.5	10.01	12.46	11.90
0.6	10.36	12.32	12.81
0.7	10.22	11.34	12.67
0.8	10.29	11.48	13.09
0.9	10.64	12.11	13.16
1.0	10.43	12.18	12.31
2.0	11.06	12.04	12.60
3.0	11.06	12.32	13.30
4.0	10.57	12.12	13.72
5.0	11.06	12.18	13.86
Check	10.57	11.13	13.24

TABLE 14

Increase in nitrogen over the nitrogen in the beginning for the different periods

AMOUNT OF STRAW ADDED	END OF 3 WEEKS	END OF 6 WEEKS
per cent	*mgm.*	*mgm.*
0.1	2.03	3.71
0.2	2.14	2.84
0.3	2.96	3.38
0.4	2.10	2.31
0.5	2.45	1.89
0.6	1.96	2.45
0.7	1.12	2.45
0.8	1.19	2.80
0.9	1.47	2.52
1.0	1.75	1.88
2.0	0.98	1.54
3.0	1.26	2.24
4.0	1.55	3.15
5.0	1.12	2.80
Check	0.56	1.77

of nitrogen over a period of 3 and 6 weeks. In one set of tests the different amounts of straw were added. Total-nitrogen determinations were made in duplicate immediately at the end of 3 and 6 weeks by the modified Gunning method. At the same time another series was conducted to which 1 per cent

of mannite had been applied in addition to the straw. From an analysis of tables 12, 13, 14 and 15, it is seen that straw serves as a source of carbon to nitrogen-fixing bacteria. In the case in which the mannite was added, more

TABLE 15

The effect of straw on nitrogen fixation; nitrogen 10 gm. of soil; mannite added—Average of two determinations

AMOUNT OF STRAW ADDED	AT BEGINNING	END OF 3 WEEKS	END OF 6 WEEKS
per cent	*mgm.*	*mgm.*	*mgm.*
0.1	10.36	13.51	14.21
0.2	10.43	13.79	14.07
0.3	10.43	13.81	13.86
0.4	10.78	13.72	13.79
0.5	10.15	13.72	13.86
0.6	10.92	13.72	13.37
0.7	10.62	13.64	14.14
0.8	10.78	13.23	14.21
0.9	10.78	14.07	14.35
1.0	10.29	13.65	14.07
2.0	10.85	13.93	14.21
3.0	11.38	13.86	14.35
4.0	10.78	14.21	15.19
5.0	10.92	14.07	14.77
Check	10.85	13.30	14.14

TABLE 16

Increase in nitrogen over the nitrogen in the beginning for the different periods; mannite added

AMOUNT OF STRAW ADDED	END OF 3 WEEKS	END OF 6 WEEKS
per cent	*mgm.*	*mgm.*
0.1	3.15	3.86
0.2	3.36	3.64
0.3	3.38	3.43
0.4	2.94	3.01*
0.5	3.57	3.71
0.6	2.80	2.67*
0.7	3.02	3.52
0.6	2.45	3.43
0.9	3.29	3.57
1.0	3.36	3.78
2.0	3.08	3.36
3.0	2.48	2.97
4.0	3.43	4.41
5.0	3.15	3.75
Check	2.45	3.29

nitrogen was fixed, mannite being a very readily utilized source of carbon. In only three cases was there less nitrogen fixed than in the check. These are marked with an asterisk in the tables.

The effect of straw on the number of bacteria in the soil

In this test straw in the varying amounts was added to tumblers containing 100 gm. of soil each. After 3 weeks the bacteria were counted by the dilution-beef-agar-plate method. Thereafter the numbers of bacteria in the soil were counted at approximately 6-week intervals over a period of 71 weeks. The initial number of bacteria in the soil was 2,730,000 per gram.

From an examination of table 17 it is at once apparent that as the straw increases bacteria increase in numbers. This is especially noticeable above 1 per cent straw, where unheard-of numbers of bacteria were present. For example, at the end of 12 weeks the count with the 5 per cent straw lies between 130 and 190 million bacteria. At the end of the 71 weeks the count with the 4 per cent straw has fallen off to 67 million but the same general relationship holds even then. The bacterial counts for all the periods are averaged and the results graphically expressed in figure 1. In this diagram the ordinate represents the average number of bacteria for the whole period of the work. From the numbers given the last four ciphers have been omitted. The abscissa represents the percentage of straw added.

The effect of straw on the types of bacteria present in the soil

In order to find out whether straw stimulates or inhibits any particular group of bacteria, a study of the effect of straw on bacteria was carried out. About one year (53 weeks) after the soil had been inoculated with the various amounts of straw as described in the preceding section, the soil was plated for counting. All the colonies on a high-dilution plate, usually one on which there were between fifty and one hundred colonies, were picked and inoculated on agar slants. The following soil samples were used: 0.3 per cent, 0.6 per cent, 1 per cent and 4 per cent straw, and the check. Besides these, the writer made a study of the bacteriology of a fresh sample of straw and of soil. It is obviously impossible to study all the bacteria in a gram of soil, but this method gives an approximation of the flora.

The following culture media were used:

1. *Litmus milk*—Dehydrated litmus milk (Digestive Ferments Company), 29 gm. in 1000 cc. of distilled water.
2. *Beef bouillon*—1 per cent peptone, 0.5 per cent salt, 0.3 per cent Liebig's extract of beef, in distilled water. Reaction +1.5 per cent.
3. *Beef peptone agar*—Same as no. 2 with 1.5 per cent agar-agar.
4. *Gelatin*—Same as no. 2 with 10 per cent gelatin, reaction +1.5 per cent.
5. *Dextrose bouillon*—Same as no. 2 with 1 per cent dextrose, reaction neutral.
6. *Lactose bouillon*—Same as no. 2 with 1 per cent lactose, reaction neutral.
7. *Dunham's solution*—1 per cent peptone, 0.5 per cent salt in distilled water.
8. *Nitrate solution*—0.1 per cent peptone, 0.02 per cent KNO_3, in distilled water.

TABLE 17

Number of bacteria per gram of soil—last four ciphers omitted

AMOUNT OF STRAW	AFTER 3 WEEKS	AFTER 8 WEEKS	AFTER 13 WEEKS	AFTER 18 WEEKS	AFTER 24 WEEKS	AFTER 30 WEEKS	AFTER 36 WEEKS	AFTER 42 WEEKS	AFTER 48 WEEKS	AFTER 53 WEEKS	AFTER 60 WEEKS	AFTER 64 WEEKS	AFTER 71 WEEKS	AVERAGE
per cent														
0.1	276	300	196	472	97	200	230	200	73	170	174	234	190	216.3
0.2	365	500	150	249	293	130	191	371	310	500	570	534	256	339.9
0.3	351	1,000	322	395	530	355	138	490	97	550	480	335	390	418.0
0.4	546	1,900	346	359	690	260	404	800	550	560	310	710	300	565.0
0.5	700	5,000	700	359	510	820	416	640	84	800	330	860	420	895.3
0.6	900	2,400	1,300	395	670	685	404	950	131	1,200	570	1,350	590	903.4
0.7	900	2,700	1,100	700	637	610	760	660	140	750	500	1,030	560	849.7
0.8	3,100	2,000	1,400	700	1,320	1,240	137	1,440	270	1,150	590	1,340	520	1146.7
0.9	5,500	3,900	1,300	954	850	1,100	670	1,280	200	1,900	870	1,050	870	1572.6
1.0	3,200	4,000	800	900	1,920	1,210	1,540	1,470	200	1,700	1,070	1,240	1,800	1615.7
2.0	2,600	3,800	3,100	2,800	5,300	2,380	2,690	5,020	730	4,000	5,000	2,820	1,780	2824.2
3.0	13,800	11,300	7,900	3,000	8,200	4,500	3,500	5,500	490	6,300	2,900	5,000	6,100	6037.7
4.0	13,400	14,200	10,000	7,700	16,000	7,730	7,200	12,800	1,920	7,800	5,000	7,900	6,700	9104.0
5.0	13,700	13,300	19,200	14,600	17,800	5,200	7,800	8,000	1,310	5,700	3,800	8,300	4,300	9462.3
Check	326	222	111	230	166	190	66	196	40	150	94	290	100	167.7

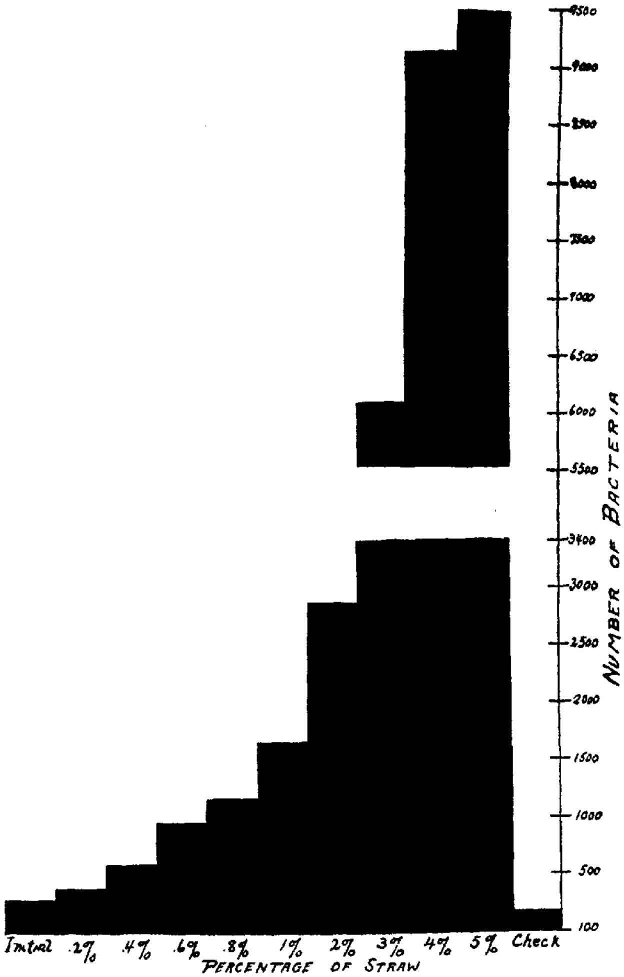

FIG. 1. DIAGRAM SHOWING NUMBERS OF BACTERIA IN SOILS VARIOUSLY TREATED

Media no. 5 and 6 were put in fermentation tubes. All media were sterilized in an autoclave 15 minutes, at 15 pounds pressure. The media were used for the following purposes:

1. *Litmus milk*—Acid and alkali production; casein coagulated or digested.
2. *Beef bouillon*—Type of growth.
3. *Agar slant*—Type of growth.
4. *Gelatin stab*—Type of liquefaction or type of growth.
5. *Dextrose bouillon*—Acid and gas production.
6. *Lactose bouillon*—Acid and gas production.
7. *Dunham's solution*—Indol and ammonia production.
8. *Nitrate solution*—Nitrite production.

The staining reactions and morphology of the bacteria were determined, as well as their physiological and cultural characters, on the above media.

In the tables giving the results the reaction in Dunham's solution are omitted because all the organisms studied formed ammonia and none formed indol. None of the bacteria formed gas in either dextrose or lactose bouillon. None of the organisms except sixteen in the sample of straw formed acid in lactose bouillon. These are marked with an asterisk in the table. The reactions in lactose bouillon are omitted from the tables.

The cultural and morphological characters were compared with descriptions in Chester's Manual of Determinate Bacteriology (2). As the descriptions are often meagre, the names assigned to bacteria in the tables are but an approximation.

From a study of tables 18 to 25 inclusive, it becomes apparent that the predominating type of bacteria in all cases is the gram positive, proteolytic, spore-bearing streptobacillus. In the field soil that had no storage 40.6 per cent are *B. subtilis* and 49.5 per cent *B. megatherium*, organisms which have the same general characters. Practically all the bacteria are gram positive, spore-bearing bacilli, as shown in the table 25. All the organisms down to *Bact. oxylactium* are of this type. In the straw there is a more varied picture. Only 27 per cent belong to the *B. subtilis* and *B. megatherium* groups, although 81 per cent of the bacteria are spore-bearing bacilli.

The straw does not seem to stimulate any group of bacteria. The *B. subtilis* type predominates in all cases and also in the check and in the field soil. There is no progressive increase or decrease in this type. Although straw stimulates bacterial reproduction, as was shown previously, it is a reproduction of the types already in the soil, and no special group is favored.

A large number of the bacteria formed nitrites from nitrates in culture tubes, 72.5 per cent with 0.3 per cent straw, 55.2 per cent with 0.6 per cent straw, 86.2 per cent with 1 per cent straw, 90 per cent with 4 per cent straw and 83 per cent with the check. It was thought that this might have some bearing on the problem. With the higher percentages of straw there are enormous numbers of bacteria, and they may form nitrites in the soil from the nitrates. With this in mind the various amounts of straw were added to

TABLE 18

Bacteria in 0.3 per cent straw treatment—40 cultures studied

NUMBER	AGAR SLANT	GELATIN STAB	DEXTROSE BOUILLON	LITMUS MILK	BEEF TEA	NITRATE SOLUTION	GRAM STAIN AND MORPHOLOGY	NAME
			acid			*nitrites*		
15	Echinulate	+ Stratiform	−	Alkaline digested	Sediment	+	Gram +, spores streptobacillus	*B. subtilis*
12	Echinulate	+ Stratiform	−	Alkaline digested	Sediment	−	Gram +, spores streptobacillus	*B. subtilis*
6	Beaded	− Filiform	−	No change	Sediment	−	Gram +, spores bacillus	*B. ginglimus*
3	Beaded	− Filiform	+	Alkaline digested	Sediment	+	Gram +, spores bacillus	*B. ginglimus*
1	Beaded	+ Nailhead	−	Alkaline digested	Sediment	+	Gram +, spores bacillus	*B. megatherium*
1	Beaded	+ Stratiform	−	Alkaline digested	Sediment	−	Gram +, coccus	*M. radiatus*
1	Echinulate	+ Stratiform	−	Alkaline digested	Sediment	+	Gram +, coccus	*M. radiatus*
1	Echinulate	+ Stratiform	−	Alkaline digested	Sediment	−	Gram +, coccus	*M. radiatus*

TABLE 19

Bacteria in 0.6 per cent straw treatment—38 cultures studied

NUMBER	AGAR SLANT	GELATIN STAB	DEXTROSE BOUILLON	LITMUS MILK	BEEF TEA	NITRATE SOLUTION	GRAM STAIN AND MORPHOLOGY	NAME
			acid			*nitrites*		
19	Echinulate	+ Stratiform	–	Alkaline digested	Sediment	+	Gram +, spores streptobacillus	*B. subtilis*
13	Echinulate	+ Stratiform	–	Alkaline digested	Sediment	–	Gram +, spores streptobacillus	*B. subtilis*
2	Beaded	– Filiform	–	No change	Sediment	–	Gram +, spores streptobacillus	*B. ginglimus*
2	Echinulate	– Filiform	+	Acid coagulated	Sediment	+	Gram +, spores streptobacillus	*B. punctiformis*
1	Beaded	– Filiform	–	No change	Sediment	–	Gram +, spores streptobacillus	*B. ginglimus*
1	Echinulate	– Growth on the surface	–	Alkaline digested	Sediment	–	Gram +, spores short bacillus	*B. siccus*

TABLE 20

Bacteria in 1.0 per cent straw treatment—65 cultures studied

NUMBER	AGAR SLANT	GELATIN STAB	DEXTROSE BOUILLON	LITMUS MILK	BEEF TEA	NITRATE SOLUTION	GRAM STAIN AND MORPHOLOGY	NAME
			acid			*nitrites*		
43	Echinulate	+ Stratiform	−	Alkaline digested	Sediment	+	Gram +, spores streptobacillus	*B. subtilis*
7	Echinulate	+ Stratiform	−	Alkaline digested	Sediment	−	Gram +, spores streptobacillus	*B. subtilis*
1	Echinulate	+ Infundibulum	−	Alkaline digested	Sediment	−	Gram +, spores streptobacillus	*B. subtilis*
5	Beaded	− Filiform	+	No change	Sediment	+	Gram +, spores short bacillus	*B. siccus*
4	Echinulate	+ Stratiform	+	Acid coagulated	Sediment	+	Gram +, spores streptobacillus	*B. liodermos*
2	Echinulate	+ Stratiform	+	Alkaline digested	Sediment	+	Gram +, spores large plump bacillus	*B. verticillatum*
1	Filiform	− Filiform	+	Alkaline digested	Sediment	+	Gram +, spores streptobacillus	*B. ginglimus*
1	Beaded	+ Infundibulum	−	No change	No change	−	Gram +, spores streptobacillus	*B. megatherium*
1	Filiform pink	− Filiform	−	Alkaline digested	Sediment	+	Gram +, bacillus	

TABLE 21

Bacteria in 4 per cent straw treatment—98 cultures studied

NUMBER	AGAR SLANT	GELATIN STAB	DEXTROSE BOUILLON	LITMUS MILK	BEEF TEA	NITRATE SOLUTION	GRAM STAIN AND MORPHOLOGY	NAME
			acid			*nitrites*		
71	Echinulate	+ Stratiform	−	Alkaline digested	Sediment	+	Gram +, spores streptobacillus	*B. subtilis*
6	Echinulate	+ Stratiform	−	Alkaline digested	Sediment	−	Gram +, spores streptobacillus	*B. subtilis*
8	Echinulate	+ Stratiform	−	No change	Sediment	+	Gram +, spores streptobacillus	*B. subtilis*
4	Echinulate	+ Stratiform	+	Alkaline digested	Sediment	+	Gram +, spores streptobacillus	*B. subtilis*
1	Echinulate	+ Stratiform	+	Alkaline digested	Sediment	−	Gram +, spores streptobacillus	*B. subtilis*
1	Echinulate	+ Stratiform	+	No change	Sediment	+	Gram +, spores streptobacillus	*B. subtilis*
1	Echinulate	+ Stratiform	−	No change	Sediment	−	Gram +, spores streptobacillus	*B. subtilis*
4	Echinulate	+ Stratiform	+	Acid	Sediment	+	Gram +, spores streptobacillus	*B. liodermos*
1	Echinulate	+ Stratiform	+	Acid	Sediment	−	Gram +, spores streptobacillus	*B. liodermos*
1	Filiform	− Filiform	−	Alkaline digested	Sediment	−	Gram +, short bacterium	

TABLE 22

Bacteria in check, no straw added—72 cultures studied

NUMBER	AGAR SLANT	GELATIN STAB	DEXTROSE BOUILLON	LITMUS MILK	BEEF TEA	NITRATE SOLUTION	GRAM STAIN AND MORPHOLOGY	NAME
			acid			*nitrites*		
32	Echinulate	+ Stratiform	–	Alkaline digested	Sediment	+	Gram +, spores streptobacillus	*B. subtilis*
24	Echinulate	+ Stratiform	+	Alkaline digested	Sediment	+	Gram +, spores streptobacillus	*B. subtilis*
3	Echinulate	+ Stratiform	–	Alkaline digested	Sediment	–	Gram +, spores streptobacillus	*B. subtilis*
4	Echinulate	+ Stratiform	+	Alkaline digested	Sediment	–	Gram +, spores streptobacillus	*B. subtilis*
1	Echinulate	+ Stratiform	+	Acid coagulated	Sediment	–	Gram +, spores streptobacillus	*B. liodermos*
1	Filiform	+ Stratiform	–	Alkaline digested	Sediment	+	Gram +, drumstick spores, bacillus	*B. sublanatus*
1	Beaded	– Filiform	–	No change	Sediment	+	Gram +, spores short bacillus	*B. siccus*
1	Beaded	– Filiform	–	Alkaline digested	Sediment	+	Gram +, spores streptobacillus	*B. ginglimus*
3	Echinulate, yellow pigment	+ Stratiform	+	Acid coagulated	Sediment	–	Gram +, short bacterium	*Bact. oxylacticum*
1	Echinulate	No growth	–	No change	Sediment	+	Gram +, coccus	*M. lactis*
1	Echinulate	+ Infundibulum	+	Acid coagulated	No change	–	Gram +, coccus	*M. coronatus*

TABLE 23

Bacteria in field soil—101 cultures studied

NUMBER	AGAR SLANT	GELATIN STAB	DEXTROSE BOUILLON	LITMUS MILK	BEEF TEA	NITRATE SOLUTION	GRAM STAIN AND MORPHOLOGY	NAME
			acid			*nitrites*		
21	Echinulate	+ Infundibulum	–	Alkaline digested	Sediment	+	Gram +, spores streptobacillus	*B. megatherium*
11	Echinulate	+ Infundibulum	+	Alkaline digested	Sediment	+	Gram +, spores streptobacillus	*B. megatherium*
15	Echinulate	+ Infundibulum	–	Alkaline digested	Sediment	–	Gram +, spores streptobacillus	*B. megatherium*
3	Echinulate	+ Infundibulum	+	Alkaline digested	Sediment	–	Gram +, spores streptobacillus	*B. megatherium*
16	Echinulate	+ Stratiform	–	Alkaline digested	Sediment	+	Gram +, spores streptobacillus	*B. subtilis*
4	Echinulate	+ Stratiform	+	Alkaline digested	Sediment	+	Gram +, spores streptobacillus	*B. subtilis*
19	Echinulate	+ Stratiform	–	Alkaline digested	Sediment	–	Gram +, spores streptobacillus	*B. subtilis*
2	Echinulate	+ Stratiform	+	Alkaline digested	Sediment	–	Gram –, spores streptobacillus	*B. subtilis*
4	Echinulate	+ Complete	–	Complete digestion	Sediment	–	Gram +, spores streptobacillus	*B. mesentericus*
2	Filiform	+ Stratiform	+	Acid	Sediment	+	Gram +, spores streptobacillus	*B. liodermos*
2	Filiform	– Filiform	+	Acid	Sediment	+	Gram +, spores streptobacillus	*B. punctiformis*
2	Echinulate	– Filiform	–	Alkaline	Sediment	+	Gram +, spores streptobacillus	*B. ginglimus*

TABLE 24

Bacteria in straw—86 cultures studied

NUMBER	AGAR SLANT	GELATIN STAB	DEXTROSE BOUILLON	LITMUS MILK	BEEF TEA	NITRATE SOLUTION	GRAM STAIN AND MORPHOLOGY	NAME
			acid			*nitrites*		
19	Filiform	− Growth on surface	−	Alkaline digested	Film sediment	−	Gram +, spores short bacillus	*B. siccus*
12	Filiform	− Growth on surface	−	Alkaline digested	Film sediment	+	Gram +, spores short bacillus	*B. siccus*
8	Echinulate	+ Infundibulum	−	Complete digestion	Sediment	−	Gram +, spores streptobacillus	*B. subtilis*
6	Echinulate	+ Stratiform	−	Alkaline digested	Sediment	+	Gram +, spores streptobacillus	*B. subtilis*
9	Echinulate	+ Complete	−	Complete digestion	Film sediment	+	Gram +, spores streptobacillus	*B. megatherium*
7	Filiform	+ Infundibulum	+	Alkaline digested	Sediment	−	Gram +, spores short bacillus	*B. detrudens*
10*	Filiform	+ Complete	+	Acid	Sediment	+	Gram +, short bacterium	*Bact. lactis*
6*	Filiform	+ Infundibulum	+	Acid	Sediment	+	Gram +, coccus	*M. simplex*
5	Filiform	+ Infundibulum	+	Alkaline	Sediment	−	Gram +, occcus	*M. radiatus*
4	Echinulate	+ Stratiform	−	Alkaline	Sediment	+	Gram +, coccus	*M. radiatus*

* Acid formed in lactose bouillon.

100-gm. samples of soil to which had been added 50 mgm. of nitrate nitrogen in the form of sodium nitrate. After a period of 1 and 2 weeks the soil was leached out with distilled water and the nitrites determined by the colorimetric method (9). Nitrites were not found present in any appreciable amounts in any case.

In no case was there a larger amount of nitrites found than in the check to which no straw had been added, and in the check to which no straw and no nitrate had been added. In many cases there were no nitrites present at all. The greatest amount of nitrite found was 0.1 mgm. per 100 gm. of soil. The nitrates that are used by these bacteria are evidently built up into organic nitrogen by the bacteria and are thrown off in the form of organic nitrogenous

TABLE 25

Percentage of various bacteria

NAMES OF BACTERIA	0.3 PER CENT STRAW	0.6 PER CENT STRAW	1 PER CENT STRAW	4 PER CENT STRAW	CHECK, NO STRAW	FIELD SOIL	STRAW
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
B. subtilis	67.5	84.2	78.5	93.9	87.5	40.6	16.5
B. megatherium	2.5		1.5			49.5	10.5
B. liodermos			6.2	5.0	1.4		
B. mesentericus						4.0	
B. sublanatus					1.4		
B. detrudens							8.1
B. verticillatum			10.7				
B. ginglimus	22.5	7.9	1.5			2.0	
B. punctiformis		5.3				2.0	
B. siccus		2.6	7.7		1.4		36.0
Bact. oxylacticum					3.9		
Bact. lactis							11.6
M. lactis					1.4		
M. coronatus					1.4		
M. simplex							7.0
M. radiatus	7.5						10.5
Not identified			1.0	1.0			

material as a waste product. In the culture tubes, the media used contained only a trace of peptone and a small amount of nitrate. There was no source of carbon. In the soil, however the straw and other organic materials are present, so that the nitrate used by the bacteria is probably thrown off in an organic form.

The effect of straw on the nitrate-forming bacteria in the soil

In connection with this problem the question arises, has straw any effect on the nitrate bacteria? Does it inhibit their growth or development and consequently the formation of nitrates?

Eight strains of nitrate bacteria were isolated. The following liquid medium (4) in distilled water was used: $MgSO_4$ 0.04 per cent, $Fe_2(SO_4)_3$ 0.04 per cent, NaCl 0.05 per cent, K_2HPO_4 0.05 per cent, Na_2CO_3 0.1 per cent, $NaNO_2$ 0.1 per cent. About 1 gm. of soil was inoculated into 500-cc. Erlenmeyer flasks containing about 150 cc. of the above sterile medium. The flasks were incubated at room temperature. They were tested for the presence of nitrates weekly. As soon as nitrates were found present, 1 cc. of the medium was transferred to some new medium. After four transfers were made 1 cc. of the medium was diluted and plated on nitrite agar. This medium was the same as given above, but for the addition of 2 per cent agar-agar (Difco). Colonies were picked from the plates and inoculated into the liquid medium again, which was tested for the presence of nitrates at intervals. If nitrates were found a pure culture of nitrate bacteria had been isolated.

In this work silica jelly, the original medium on which Winogradski isolated the nitrate bacteria, was tried but much better results were obtained when agar-agar (Difco) was used as a solidifying agent. A very interesting fact was noticed in the isolation work. The nitrate bacteria formed small indentations in the agar. The agar is probably softened by the nitric acid that is formed. One organism has been isolated that completely liquefies the agar medium after a few weeks. The area about the colonies of nitrate bacteria in the agar medium also is greatly softened.

The effect of cellulose was tried on the eight strains of nitrate bacteria, first, the cellulose in filter paper, and then the cellulose in straw.

A solution of hydrated cellulose from filter paper was made up as follows (8): To a mixture of 100 cc. of concentrated H_2SO_4 and 60 cc. of water that had been cooled to 60°C., was added 5 gm. of moist filter paper. The 3-litre flask containing the mixture was shaken until the paper dissolved, and was then completely filled with cold water. The cellulose was thrown out of solution in a finely divided mass, and after being allowed to settle, the supernatant fluid was poured off. More water was added and the process repeated until all the acid disappeared. The suspension of cellulose was then made up to any strength desired. The cellulose from the straw was made in the same manner.

Various amounts of the concentrated suspension of cellulose from filter paper and straw were placed in petri dishes so that the amounts ran from 0.1 to 1 per cent at intervals of tenths, and from 1 to 5 per cent at intervals of units. In the case of straw it was found impossible to make 4 and 5 per cent suspensions of cellulose with the medium. To all petri dishes was added 10 cc. of the nitrite agar mentioned before.

The nitrate bacteria were streaked over the surface of the agar and the growth examined after intervals. There was no harmful effect caused by the cellulose on the nitrate bacteria that could be noticed. In fact with the cellulose from the filter paper the growth seemed to increase with the con-

centration. With the cellulose from straw no difference in growth could be noticed. The results of this experiment indicate that cellulose itself has no harmful effect on nitrate-forming bacteria.

SUMMARY

1. The addition of straw has a harmful effect on nitrate accumulation in the soil after a given time.
2. As the amount of straw increases the harmful effect increases.
3. Total-nitrogen determinations throughout the work show that if there is a loss in nitrate nitrogen, it is transformed to some other form of nitrogen. It is not lost to the soil.
4. Straw has an effect on nitrates already in the soil. As the straw increases the loss in nitrates increases.
5. Straw acts as a source of energy for nitrogen-fixing bacteria. The amount of nitrogen fixed is not dependent upon the amount of straw.
6. The process of ammonification is inhibited above 0.9 per cent straw. As the straw increases the harmful effect increases.
7. Straw has a decided effect on the number of bacteria. As the straw increases the bacteria increase in numbers.
8. Straw has no effect on the kind of bacteria present in the soil. The same types of bacteria predominate in straw-treated soil as predominate in soil without straw.
9. Nitrates that are used by the bacteria are not given off in the nitrite form but probably as organic nitrogen.
10. Cellulose from either filter paper or straw has no effect on the nitrate-forming bacteria.

CONCLUSION

Straw applied to the soil stimulates the reproduction of bacteria. The bacteria use the straw as a source of carbon and use the nitrates (or in some cases ammonium sulfate) as a source of nitrogen. The nitrates are transformed to organic nitrogenous material and for the time being are lost as available plant-food. The intensity of the reaction depends upon the amount of straw.

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THE SOLUBILITY OF ANIONS IN ALKALI SOILS[1]

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INTRODUCTION

Cameron and Patten (4) showed in 1906 that sodium chloride is more easily removed from soils by leaching than sodium carbonate. Their studies were made with a fine sandy loam and a silty loam soil, each of which contained considerable amounts of the sulfate, chloride and carbonate of sodium. Columns of soil were leached under constant water pressure for a period of several weeks. The volume of water that passed through the soil and its content of chloride, carbonate and bicarbonate were determined at successive intervals.

The chloride was completely removed from each soil within a few weeks. Normal carbonate also disappeared, although much more slowly than the chloride, while small amounts of bicarbonate were still present in the percolates at the close of the experiment.

In preliminary leaching experiments with a light sandy soil that contained excessive amounts of various sodium salts, we have found that both the chloride and sulfate may be readily leached out, but appreciable amounts of soluble carbonate remained in the soil after prolonged leaching.

Vinson and Catlin (14), Hare (7) and Pittman (12) have shown that increasing amounts of carbonate are dissolved when alkali soils are extracted with increasing proportions of water. As pointed out by them, the ratio of soil to water used by the different chemical laboratories to extract the salts from alkali soils, varies from 1:2 to 1:20. The soil and water are also allowed to remain in contact, usually with periodic shaking, for different lengths of time, varying from 20 minutes to 24 hours.

It is well known that certain alkali soils may be substantially improved by drainage, especially when the drainage is followed by flooding or heavy irrigation. By this means barren alkali wastes, in certain localities, have been transformed into highly productive soil. On the other hand, drainage, even when accompanied by heavy flooding, has not proved to be an adequate means of reclaiming certain tracts of black-alkali soil in California (15) and other states (6).

[1] Paper No. 78, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

From these and other references that could be cited, it seems that alkaline salts produce effects on soils, different either in kind or degree, from those produced by neutral salts. As is well known, the claim has frequently been made that alkaline salts are adsorbed by soil to a greater degree than neutral salts.

Despite the many investigations that have been made on alkali soils by the Bureau of Soils, Hilgard and various other workers, much still remains to be determined. It is desirable that we extend our knowledge concerning the mutual relationships that exist between soluble salts and soils.

In a previous paper from this laboratory (10) we have discussed some of the chemical effects that are produced when salts are added to soils. The present paper will deal mainly with the solubility of sodium salts as they occur in natural alkali soils. We have investigated (a) the effect of time on the extraction of salts, (b) the effect of different ratios of soil to water, and (c) the rate of solution of the several anions present in three types of alkali soil.

METHODS

Samples of air-dried soil were used that had been thoroughly mixed and passed through a 2-mm. screen. The extracts were made by shaking, with a mechanical shaker, portions of soil with distilled water from which all but traces of carbon dioxide had been removed by aeration. The extracts were filtered through Pasteur-Chamberland tubes with the aid of compressed air. The first portion of the filtrate, usually about 200 cc., was always discarded.

Carbonate and bicarbonate were determined as soon after filtration as possible by double titration with 0.05 N H_2SO_4, phenolphthalein and methyl orange being used as indicators. The pH values of the extracts were determined colorimetrically, with Clark and Lubs indicators and buffer solutions. Chloride was determined volumetrically by the Mohr method and sulfate gravimetrically, after first removing the silica. Nitrate was determined by reduction with aluminum in those extracts which contained appreciable amounts of chloride or dissolved organic matter. Otherwise, the nitrate was determined by the phenoldisulfonic acid method. Total soluble solids were determined by evaporating 100 cc. to dryness and heating the residue for an hour in an oven at 110°C.

The soluble constituents of the soils used in this investigation are composed mainly of sodium salts, but the content of chloride, sulfate, nitrate and soluble carbonate varies considerably. Sample 905 is a light sandy soil from Riverside, California; no. 907 is a sandy loam from Edison, California; and no. 908 is a fine sandy loam from Fresno, California. Soils 905 and 907 contain large amounts of $CaCO_3$, while soil 908 is almost free from $CaCO_3$.

EFFECT OF CO_2 IN THE COMPRESSED AIR

Since compressed air is commonly used in filtering soil extracts, it is desirable to determine whether compressed air produces any important effect on the carbonate content of the extract. It seemed possible that the carbon-dioxide content of the compressed air might convert a portion of the dissolved carbonate into bicarbonate.

Duplicate portions of several different soils, each of which contained considerable amounts of soluble carbonate, were shaken an hour with aerated water in the ratio of 1:5. One solution of each soil was filtered with the aid of compressed air in the usual way. The other portion was simultaneously filtered with the same kind of filtering apparatus, suction being used instead of compressed air. In this case, the upper end of the filtering chamber, instead of being attached to the compressed-air line, was connected with a tube filled with soda lime to protect the extract from external CO_2, the lower end of the filter being connected with a suction flask.

Analysis of the extracts showed that in no case was the content of normal carbonate reduced by filtering under pressure. On the contrary, the extracts from certain soils were found to contain slightly higher amounts of carbonate and correspondingly smaller amounts of bicarbonate, when filtered by means of compressed air, than when filtered by means of suction.

It is well known that equilibrium may be affected by pressure and temperature, and that the equilibrium between carbonate and bicarbonate is easily disturbed (3). It is possible that increasing the pressure tends to force the equilibrium of alkali soil extracts in the direction of increased amounts of CO_3. From these results it seems safe to conclude, however, that the carbon dioxide contained in ordinary compressed air does not affect the content of normal carbonate in soil extracts to any great extent.

EFFECT OF TIME ON THE SOLUBILITY

Portions of each soil were shaken with aerated water in the ratio of 1:5 for periods ranging from 5 minutes to 8 hours. The solutions were filtered immediately and analyzed. The average results of duplicate determinations are submitted in tables 1, 2 and 3.

The data show that soil 905 contains approximately 1.15 per cent total soluble matter; soil 907 contains slightly less than 0.20 per cent, and soil 908 contains approximately 2.50 per cent.

Similar results were obtained with each soil, so far as the effect of the time of shaking is concerned. The amount of total solids dissolved by shaking for 5 minutes was less than was dissolved by shaking for longer periods, but the solubility of no single ion was consistently increased in the same way.

These results indicate that constant shaking for a period of 1 hour affords sufficient time for approximate equilibrium to be established between water

and these soils. It seems probable that the variations in the lengths of time employed by the different laboratories in the extraction of alkali salts is a matter of no very great practical importance.

TABLE 1

Effect of time of shaking on the solubility of soil 905

	TIME OF SHAKING				
	5 minutes	30 minutes	1 hour	4 hours	8 hours
Carbonate (CO_3) (p.p.m.)	135	172	176	202	198
Bicarbonate (HCO_3) (p.p.m.)	263	232	247	262	385
Chloride (Cl) (p.p.m.)	2,244	2,249	2,293	2,251	2,256
Sulfate (SO_4) (p.p.m.)	4,194	4,202	4,209	4,235	4,237
Nitrate (NO_3) (p.p.m.)	326	321	321	332	332
Total soluble solids (p.p.m.)	11,065	11,392	11,577	11,650	11,782
pH value	9.0	9.2	8.7	9.0	9.0

TABLE 2

Effect of time of shaking on the solubility of soil 907

	TIME OF SHAKING				
	5 minutes	30 minutes	1 hour	4 hours	8 hours
Carbonate (CO_3) (p.p.m.)	75	90	67	63	93
Bicarbonate (HCO_3) (p.p.m.)	412	427	468	472	453
Chloride (Cl) (p.p.m.)	37	37	36	37	36
Sulfate (SO_4) (p.p.m.)	237	282	276	303	307
Nitrate (NO_3) (p.p.m.)	27	28	28	28	29
Total soluble solids (p.p.m.)	1,465	1,702	1,705	1,745	1,865
pH value	8.5	8.5	8.4	8.4	8.5

TABLE 3

Effect of time of shaking on the solubility of soil 908

	TIME OF SHAKING				
	5 minutes	30 minutes	1 hour	4 hours	8 hours
Carbonate (CO_3)* (p.p.m.)	2,853	2,868	2,820	2,880	2,868
Chloride (Cl) (p.p.m.)	7,601	7,694	7,889	7,783	7,800
Sulfate (SO_4) (p.p.m.)	1,672	1,663	1,647	1,658	1,672
Nitrate (NO_3) (p.p.m.)	1,271	1,348	1,425	1,387	1,371
Total soluble solids (p.p.m.)	25,490	25,487	27,002	26,552	26,477
pH value	9.8+	9.8+	9.8+	9.8+	9.8+

* Bicarbonate (HCO_3) was not determined because of the dark color of the extracts.

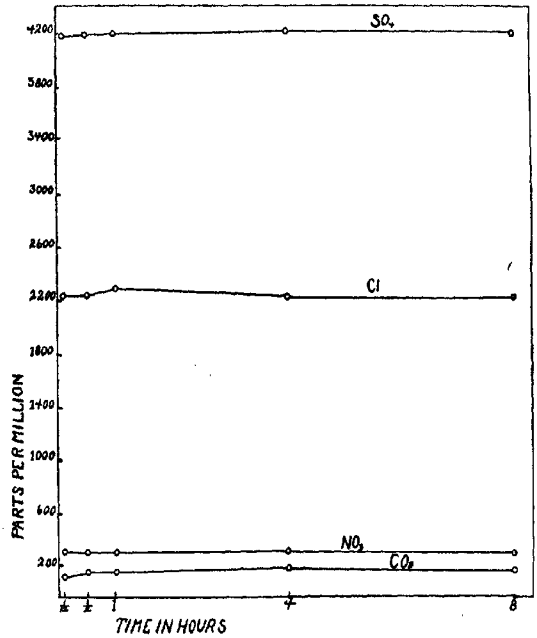

FIG. 1. EFFECT OF TIME OF SHAKING ON THE EXTRACTION OF ANIONS IN SOIL 905

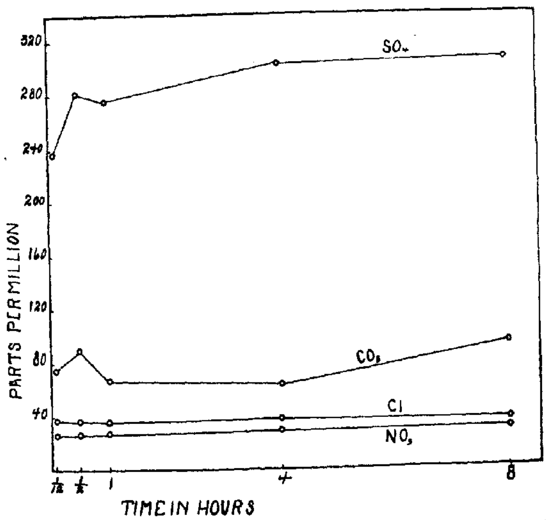

FIG. 2. EFFECT OF TIME OF SHAKING ON THE EXTRACTION OF ANIONS IN SOIL 907

EFFECT OF THE RATIO OF SOIL TO WATER

In this experiment the ratio of soil to water was varied from 1:2 to 1:80. The soil, after adding the desired amount of water, was shaken 1 hour. Duplicate determinations were made, the average results of which are submitted in tables 4, 5 and 6.

The amounts of chloride and nitrate found were reasonably uniform throughout the entire range of dilutions used. With soils 905 and 907 the amounts

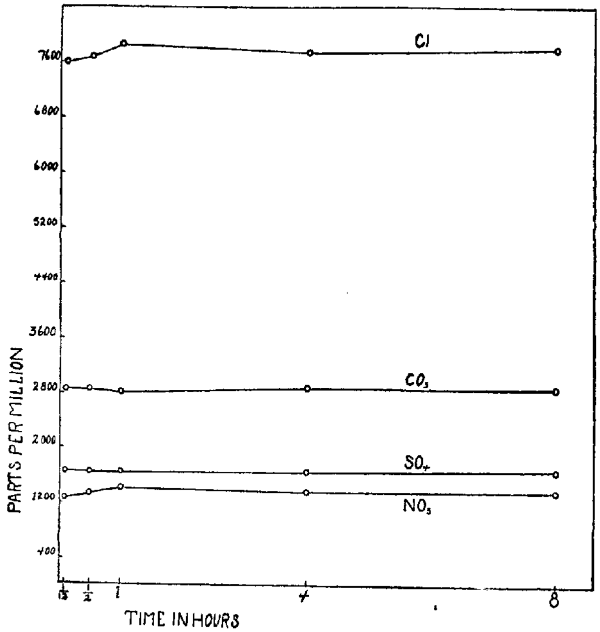

FIG. 3. EFFECT OF TIME OF SHAKING ON THE EXTRACTION OF ANIONS IN SOIL 908

of sulfate and total solids that were dissolved, advanced with increases in the proportions of water used, while with soil 908 no differences were found.

On the other hand, the data show at once that the total amounts of carbonate and bicarbonate extractable from soils 905 and 907 depend on the proportion of soil to water used. With soil 905 the amounts of carbonate and bicarbonate advanced with each increase in the proportion of water used. With soil 907 the amount of bicarbonate also increased in a similar manner, but the maximum amount of normal carbonate was recovered by the use of the ratio of 1:40.

TABLE 4

Effect of different ratios of soil to water on the solubility of soil 905

	RATIO OF SOIL TO WATER					
	1:2	1:5	1:10	1:20	1:40	1:80
Carbonate (CO_3) (p.p.m.)	73	172	292	510	780	1,080
Bicarbonate (HCO_3) (p.p.m.)	190	247	388	610	793	1,464
Total CO_3* (p.p.m.)	260	415	673	1,110	1,560	2,519
Chloride (Cl) (p.p.m.)	2,300	2,293	2,287	2,296	2,305	2,553
Sulfate (SO_4) (p.p.m.)	4,048	4,209	4,249	4,299	4,389	4,751
Nitrate (NO_3) (p.p.m.)	319	321	310	354	319	312
Total soluble solids (p.p.m.)	11,185	11,577	12,165	13,220	14,440	16,720
pH value	8.3	8.7	9.2	9.2	9.2	8.6

* Calculated from the methyl orange titration.

TABLE 5

Effect of different ratios of soil to water on the solubility of soil 907

	RATIO OF SOIL TO WATER					
	1:2	1:5	1:10	1:20	1:40	1:80
Carbonate (CO_3) (p.p.m.)	9	67	180	360	480	360
Bicarbonate (HCO_3) (p.p.m.)	294	468	640	732	976	1,830
Total CO_3* (p.p.m.)	298	527	809	1,080	1,439	2,159
Chloride (Cl) (p.p.m.)	41	36	35	35	35	35
Sulfate (SO_4) (p.p.m.)	264	276	289	277	279	361
Nitrate (NO_3) (p.p.m.)	28	28	29	32	34	28
Total soluble solids (p.p.m.)	1,161	1,705	2,225	2,550	3,140	3,800
pH value	8.0	8.4	8.8	9.3	9.4	8.6

* Calculated from the methyl orange titration.

TABLE 6

Effect of different ratios of soil to water on the solubility of soil 908

	RATIO OF SOIL TO WATER					
	1:2	1:5	1:10	1:20	1:40	1:80
Carbonate (CO_3) (p.p.m.)	2,976	2,820	2,872	2,805	2,820	2,880
Bicarbonate (HCO_3) (p.p.m.)						4,392
Chloride (Cl) (p.p.m.)	7,772	7,889	7,951	8,139	7,960	7,943
Sulfate (SO_4) (p.p.m.)	1,708	1,647		1,696	1,637	1,657
Nitrate (NO_3) (p.p.m.)	1,506	1,425	1,457	1,484	1,488	1,488
Total soluble solids (p.p.m.)	26,926	27,002	26,015	28,560	27,820	26,640
pH value	9.8+	9.8+	9.8+	9.8+	9.8+	9.4+

While bicarbonate was not determined in the extracts from soil 908 on account of the large amount of dissolved organic matter which they contained, it is nevertheless interesting to note that the amount of normal carbonate found was approximately the same with every ratio of soil to water that was used.

As is well known, ordinary distilled water contains more or less dissolved CO_2, but the amount is subject to considerable variation. In order to eliminate variation as far as possible, we, as stated above, have used distilled water which was as nearly free from CO_2 as practicable. It is of interest to note in this connection that the CO_3 content of other extracts of soil 905 have been found to be approximately the same, whether the solutions were made

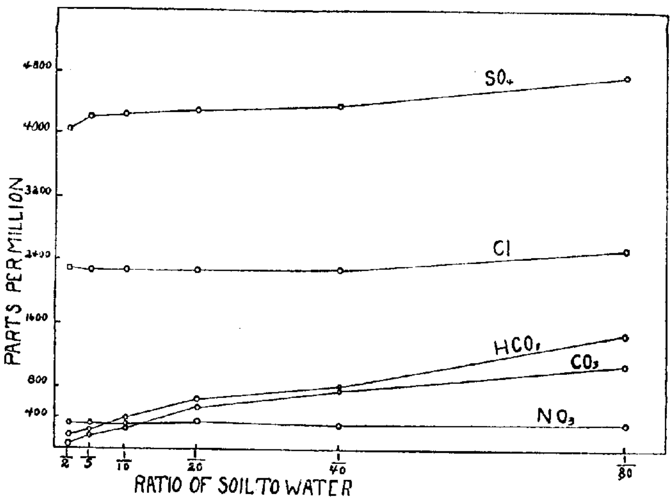

FIG. 4. EFFECT OF VARIOUS RATIOS OF SOIL TO WATER ON THE EXTRACTION OF ANIONS IN SOIL 905

with ordinary distilled water or with aerated water, but that the HCO_3 content was appreciably higher when ordinary distilled water was used. This seems to indicate that the solid phase from which the dissolved carbonate was derived was not exhausted by the amount of aerated water used.

Equilibrium between CO_3 and HCO_3 is readily disturbed by changes in the temperature, by variations in the concentration of other salts, and perhaps most of all by the presence of dissolved CO_2. Moreover, aqueous solutions prepared by dissolving either Na_2CO_3 or $NaHCO_3$ always contain both CO_3 and HCO_3 ions when equilibrium is established (3). For these reasons we have calculated the total CO_3 from the methyl-orange titrations. The results indicate that the solid phase from which the CO_3 and HCO_3 were derived was

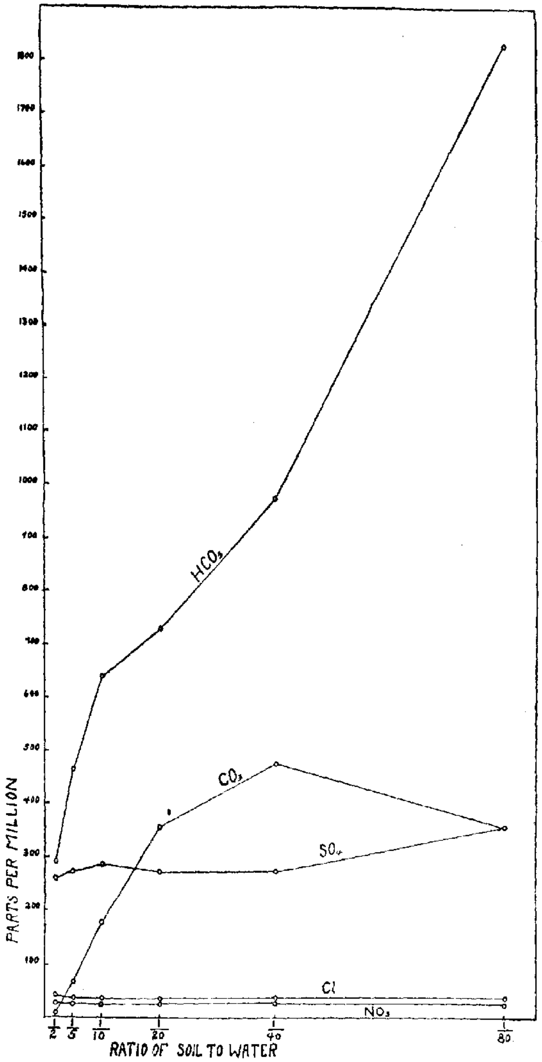

FIG. 5. EFFECT OF VARIOUS RATIOS OF SOIL TO WATER ON THE EXTRACTION OF ANIONS IN SOIL 907

not exhausted in soils 905 and 907, even by using 80 times as much water as soil.

The fact that soils 905 and 907 contain relatively large amounts of $CaCO_3$, while soil 908 contains practically none, must be taken into consideration in this connection. When expressed on the basis of air-dried soil, the 1:2 extract of soil 905 was found to contain 26 parts per million of calcium. With each increase in the proportion of water above the ratio of 1:2, greater absolute amounts of calcium were dissolved, the 1:80 extract having been found to

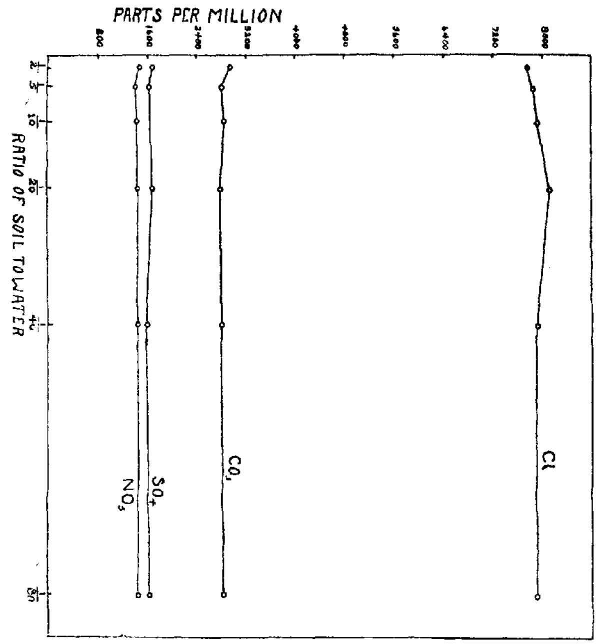

FIG. 6. EFFECT OF VARIOUS RATIOS OF SOIL TO WATER ON THE EXTRACTION OF ANIONS IN SOIL 908

contain 528 parts per million of calcium. The absolute amount of dissolved magnesium also increased with dilution, but to a lesser degree than that of calcium. On the other hand, the total amount of dissolved calcium and magnesium was by no means sufficient to satisfy the CO_3 and HCO_3 content of any of the extracts. In fact, the amount of sodium which passed into solution from adsorbed substances, or more probably from compounds of low solubility, increased with dilution very much more rapidly than was the case with calcium and magnesium. Since $CaCO_3$ is appreciably soluble in

water and very much more so in solutions of neutral salts, and in view of the amounts of dissolved calcium, it is reasonably certain that a part of the CO_3 and HCO_3 found in the extracts of soils 905 and 907 was derived from $CaCO_3$, but it is certain that a still larger part of the dissolved CO_3 and HCO_3 was derived from sodium compounds.

The OH-ion concentration of the extracts is especially interesting. It will be noted that within certain limits of dilution, soils 905 and 907 gave extracts the pH value of which increased (denoting increasing OH-ion concentrations) as the proportion of water to soil increased.

With soil 905 the pH value of the extracts increased from 8.3 to 9.2 as the proportion of water was increased from 1:2 to 1:10, and further dilution up to 1:40 gave solutions of the same pH value as the 1:10 solution.

The 1:2 extract of soil 907 gave a pH value of 8.0 and each increase in the proportion of water, until the ratio of 1:40 was reached, gave extracts with increasing OH-ion concentration. With this dilution the pH value was 9.4.

TABLE 7

The concentration of CO_3 and HCO_3 in soil extracts

	RATIO OF SOIL TO WATER					
	1:2	1:5	1:10	1:20	1:40	1:80
	p. p. m.	*p. p. m*	*p. p. m.*	*p. p. m.*	*p. p. m*	*p. p. m.*
Soil 905:						
CO_3	36.5	34.0	29.0	25.5	19.5	13.5
HCO_3	95.0	49.0	39.0	30.5	20.0	18.0
Soil 907:						
CO_3	4.5	13.0	18.0	18.0	12.0	4.5
HCO_3	147.0	94.0	64.0	37.0	24.0	23.0
Soil 908:						
CO_3	1488.0	564.0	287.0	140.0	70.5	36.0

The extracts of soil 908 obtained by the use of ratios ranging from 1:2 to 1:40 each gave a pH value of 9.8 or more, which is the upper limit of the standard buffer solutions at our disposal. The 1:80 extract, however, was less alkaline, having a pH value of 9.4. In view of the fact that this soil contains relatively large amounts of sodium carbonate, it is not surprising that the OH-ion concentration of each of its extracts was high.

The preceding data were calculated as parts per million of the air-dried soil. In making the determinations it was noted, in the case of one soil, that the solutions obtained with the use of relatively small proportions of water actually contained less normal carbonate per unit volume than the 1:20 extract. To bring out the effects of dilution more clearly, the content of CO_3 and HCO_3 expressed as parts per million of the extract is submitted in table 7.

It will be noted that the 1:2 and 1:5 extracts of soil 905 contained approximately equal concentrations of CO_3. With each succeeding increase in the

relative proportions of water above 1:5, the extracts contained somewhat lower concentrations of CO_3, but the decreases were not proportional to the increases in the amount of water used. With soil 907 the concentration of CO_3 in the extracts increased substantially as the proportion of water was increased, reaching its maximum with the ratios of 1:10 and 1:20. On the other hand, soil 908 gave extracts whose content of CO_3 was approximately inversely proportional to the amount of water used.

The HCO_3 content of the extracts of soils 905 and 907 was found to decrease as the proportion of water was increased, but in view of the variation in CO_3 already noted the ratio of CO_3 to HCO_3 must have varied.

These data are in harmony with the results of Cameron and Briggs (3) in showing that the equilibrium between CO_3 and HCO_3 is affected by concentration. But, as suggested above, shifts in the equilibrium may also take place as a result of changes in the temperature and the partial pressure of CO_2. As the temperature is increased, or as the partial pressure of CO_2 is decreased, the relative proportion of CO_3 will increase.

Since the concentration of salts in alkali soils is subject to wide fluctuation as a result of irrigation, rains and evaporation, and the temperature of the soil and air varies many degrees within a single 24-hour period, and since the amount of CO_2 is also subject to change, it seems questionable whether there is anything to be gained by making a distinction between CO_3 and HCO_3 in the ordinary analysis of alkali soils. Further discussion on this point will be submitted elsewhere.

It is quite evident from the preceding data that the amount of soluble carbonate and bicarbonate that will be found in a black-alkali soil may be influenced considerably by the ratio of soil to water used in making the extract. With certain soils the wider the ratio the greater will be the total amount found, while with other soils this does not seem to be true.

The preceding data indicate that the OH-ion concentration of an alkali soil, when determined in an aqueous extract or suspension of the soil, may, in some cases at least, be very different from that of the soil solution as it occurs in the open field. Under such conditions the OH-ion concentration may not be above the toxic limit for plant growth (8), although the soil may actually contain appreciable amounts of sodium carbonate.

The occurrence of OH-ions in excess of H-ions in the soil moisture is mainly due to the hydrolysis of salts of strong bases and weak acids. The preceding data indicate that both the hydrolysis and the solubility of sodium carbonate in soil 907 are dependent, to a considerable extent, on the proportion of soil to water; that is, on the total concentration of the solution at equilibrium with the solid phase.

Not only is the occurrence of excessive alkalinity in the soil solution due to hydrolysis, mainly of carbonates, but the actual concentration of OH ions at a given instant is dependent on the partial pressure of carbon dioxide in the soil atmosphere. Since the latter probably varies from time to time, depend-

ing on the amount furnished by growing plants and the variable activity of the micro-organisms present, the OH-ion concentration must vary also.

While a determination of the pH value of a soil suspension or extract, as an isolated soil factor, seems to be of doubtful value, practically every student of the alkali problem has placed emphasis on the question of the total amount of sodium carbonate present.[2] The preceding data show, however, that the usual methods do not give a true measure of the total amount present. In a subsequent paper, other methods for the determination of black alkali and the importance of carbon dioxide as a neutralizing agent in alkali soils, will be discussed.

RATE OF SOLUTION OF ANIONS IN ALKALI SOILS

As a means of securing further light on the solubility of the different constituents of these soils, portions of each were extracted with water several times. The procedure adopted was as follows.

Weighed amounts of soil were placed in clean acid bottles and sufficient water was added to effect three different ratios of soil to water. After shaking for 1 hour the solutions were filtered through Pasteur-Chamberland filters, care being taken to transfer as much of the soil as possible to the filter chamber.

The filtration was continued until all of the free water had been forced through the filter tube. The remaining soil was removed from the filtering apparatus by the aid of a spatula and re-introduced into the original bottle. A second quantity of water was added, sufficient in amount to effect the same ratio as before, and the contents were shaken for 1 hour and filtered. This process was repeated twice with soils 905 and 907 and three times with soil 908. The successive filtrates were analyzed with the results shown in tables 8, 9 and 10.

It will be noted that when soil 905 was extracted a second time with water in the ratio of 1:2 or 1:5, solutions were obtained which contained as much CO_3 and HCO_3 as the first extracts. With those ratios both the second and third extracts of soil 907 contained greater amounts of CO_3 than the first, while the second extraction with the 1:10 ratio yielded as much CO_3 as the first.

In contrast to soils 905 and 907, a relatively large proportion of the CO_3 in soil 908 was dissolved by the first extraction. It is interesting to note, however, that appreciable amounts of soluble CO_3 still remained in this soil after it had been extracted three times. In other words, the absolute amount of sodium carbonate that remained undissolved after the first extraction was appreciable in each of these soils, although not very great in any one of them.

[2] It is well known that successful crops may sometimes be grown on soils that contain several hundred parts per million of soluble CO_3. The pH value of a suspension of such soils is frequently above 9.0, but owing to adsorption and the action of CO_2, the soil films that are in contact with the roots of the growing plants probably contain much lower OH-ion concentration than the soil mass as a whole.

TABLE 8

Constituents dissolved from soil 905 by successive extraction

	RATIO OF SOIL TO WATER								
	1:2			1:5			1:10		
	First extraction	Second extraction	Third extraction	First extraction	Second extraction	Third extraction	First extraction	Second extraction	Third extraction
Carbonate (CO_3) (p.p.m.)	72	75	39	168	189	90	277	172	30
Bicarbonate (HCO_3) (p.p.m.)	201	464	354	255	610	400	396	892	678
Chloride (Cl) (p.p.m.)	2,300	268	53	2,296	93	9	2,278	66	9
Sulfate (SO_4) (p.p.m.)	3,964	540	107	4,218	192	41	4,239	138	56
Nitrate (NO_3) (p.p.m.)	314	46	5	321	22	3	300	16	0
Total soluble solids (p.p.m.)	11,134	2,257	1,006	11,702	1,750	882	11,970	1,965	1,215
pH value	8.4	9.3	8.6	8.6	9.4	8.4	9.0		8.2

TABLE 9

Constituents dissolved from soil 907 by successive extraction

	RATIO OF SOIL TO WATER								
	1:2			1:5			1:10		
	First extraction	Second extraction	Third extraction	First extraction	Second extraction	Third extraction	First extraction	Second extraction	Third extraction
Carbonate (CO_3) (p.p.m.)	9	28	33	67	94	63	180	180	45
Bicarbonate (HCO_3) (p.p.m.)	294	233	211	468	327	251	640	320	358
Chloride (Cl) (p.p.m.)	41	0	0	36	0	0	35	0	0
Sulfate (SO_4) (p.p.m.)	264	73	40	276	57	26	289	89	32
Nitrate (NO_3) (p.p.m.)	28	2	0	28	3	0	29	0	0
Total soluble solids (p.p.m.)	1,161	588	508	1,705	955	750	2,225	1,175	780
pH value	8.0	8.5	8.3	8.4	8.9	8.2	8.8	9.0	8.2

TABLE 10

Constituents dissolved from soil 908 by successive extraction

	RATIO OF SOIL TO WATER									
	1:5				1:10				1:20	
	First extraction	Second extraction	Third extraction	Fourth extraction	First extraction	Second extraction	Third extraction	Fourth extraction	First extraction	Second extraction
Carbonate (CO_3) (p.p.m.)	2,820	202	120	60	2,872	390	120	30	2,805	375
Bicarbonate (HCO_3) (p.p.m.)			369	289		778	404	290		1,220
Chloride (Cl) (p.p.m.)	7,889	197	9	0	7,951	124	9	0	8,139	159
Sulfate (SO_4) (p.p.m.)	1,647	87	0	0		92	20	0	1,696	36
Nitrate (NO_3) (p.p.m.)	1,425	26	13	9	1,457	18	11	7	1,484	7
Total soluble solids (p.p.m.)	27,002	2,300	925	505	26,015	2,100	1,010	500	28,560	2,020
pH value	9.8+	9.2	8.7		9.8+	9.2	9.0	8.5	9.8+	8.7

The major portion of the chloride, sulfate and nitrate was removed from each soil by the first extraction. In fact, the quantities of these ions found in each succeeding extract was such as may reasonably be supposed to have

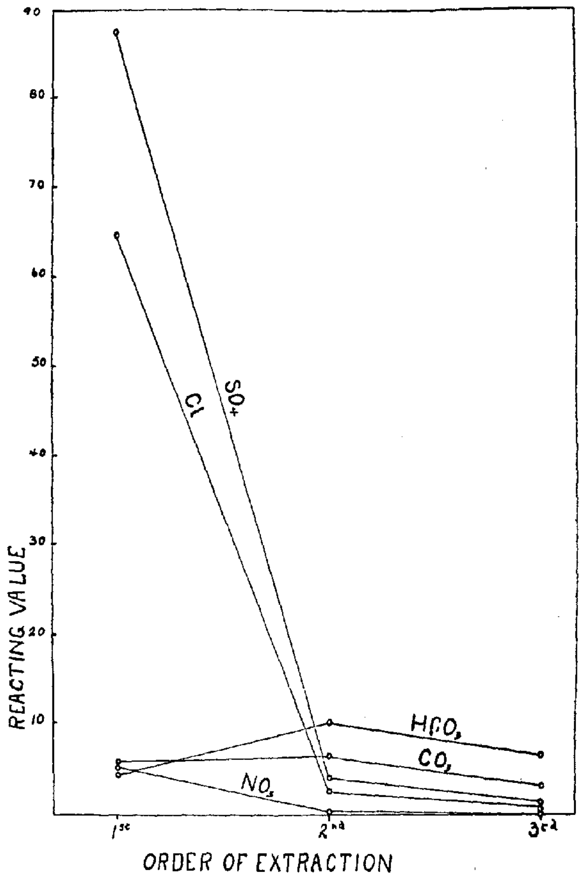

FIG. 7. CONCENTRATION OF THE VARIOUS ANIONS IN SUCCESSIVE EXTRACTS (1:5) OF SOIL 905

been derived from the adhering moisture remaining from the immediately preceding extraction.

Since the final extract from each soil still showed the presence of soluble carbonate, a sample of soil 853, which was obtained from the same locality

as soil 905, was shaken six successive times with fresh portions of water, at the ratio of 1:5. Carbonate, bicarbonate, chloride and pH values were determined in the filtrates as shown in table 11.

It will be noted that the second extract of this soil also contained greater amounts of CO_3 than the first, but that, while the carbonate content of the succeeding extracts gradually decreased, small amounts still remained after the fifth extraction. The bicarbonate slowly decreased with each extraction,

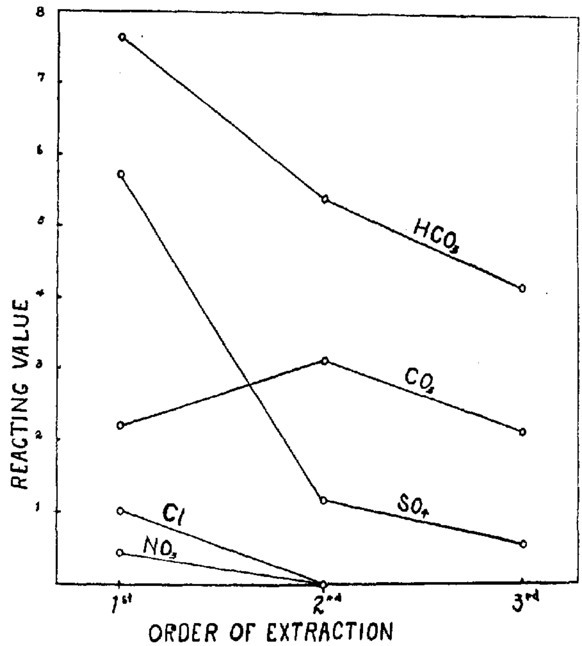

FIG. 8. CONCENTRATION OF THE VARIOUS ANIONS IN SUCCESSIVE EXTRACTS (1:5) OF SOIL 907

but considerable amounts still remained at the end of the experiment. The chloride, on the other hand, was completely removed by the second extraction.

The relative solubility of the anions in these soils becomes more apparent from a study of the data presented in tables 12, 13 and 14. In these tables are recorded the reacting values of the anions computed from the data reported in tables 8, 9 and 10. The calculations were made by multiplying the parts per million of a given ion by its reaction coefficient.[3]

[3] The reacting coefficient of an ion is the reciprocal of its weight on the atomic scale divided by its valency.

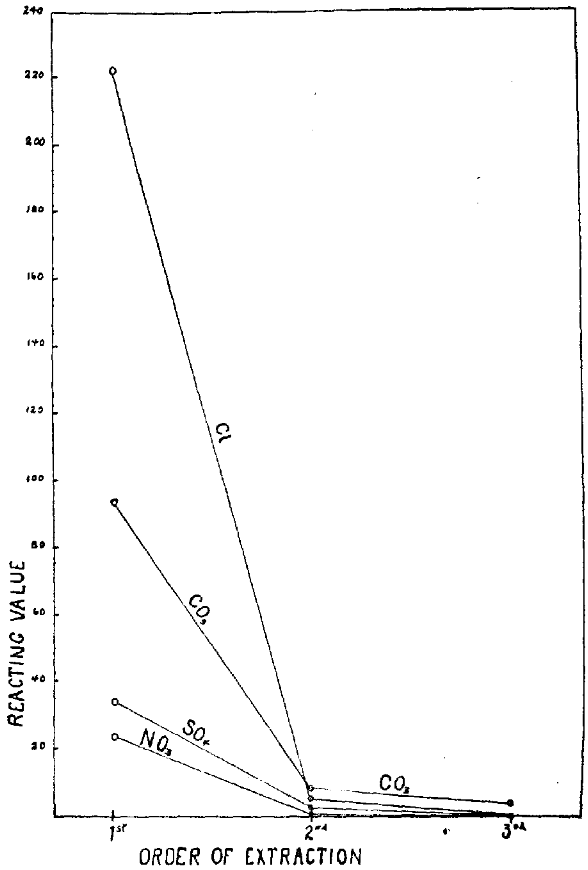

FIG. 9. CONCENTRATION OF THE VARIOUS ANIONS IN SUCCESSIVE EXTRACTS (1:5) OF SOIL 908

TABLE 11

Results of repeated extraction with soil 853

	ORDER OF EXTRACTION					
	First extraction	Second extraction	Third extraction	Fourth extraction	Fifth extraction	Sixth extraction
Carbonate (CO_3) (p.p.m.)	75	112	52	30	22	15
Bicarbonate (HCO_3) (p.p.m.)	655	625	495	472	456	442
Chloride (Cl) (p.p.m.)	1,901	159	0	0	0	0
pH value	8.8	8.8	8.8	8.5	8.4	8.0

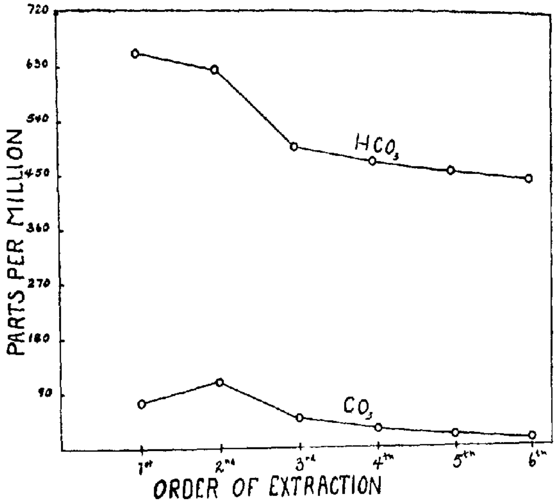

FIG. 10. AMOUNT OF CO_3 AND HCO_3 REMOVED FROM SOIL 853 BY REPEATED EXTRACTION WITH WATER

TABLE 12

Anions dissolved from soil 905 by successive extraction

	RATIO OF SOIL TO WATER								
	1:2			1:5			1:10		
	First extraction	Second extraction	Third extraction	First extraction	Second extraction	Third extraction	First extraction	Second extraction	Third extraction
	Reacting values								
CO_3	2.40	2.50	1.30	5.59	6.29	3.00	9.22	5.73	1.00
HCO_3	3.29	7.61	5.80	4.18	10.00	6.56	6.49	14.63	11.12
Cl	64.86	7.56	1.49	64.75	2.62	0.25	64.24	1.86	0.25
SO_4	82.45	11.23	2.22	87.73	3.99	0.85	88.17	2.87	1.16
NO_3	5.05	0.74	0.08	5.17	0.35	0.05	4.83	0.26	0
	Reacting value in percentage								
CO_3	0.76	4.22	5.97	1.67	13.53	14.01	2.66	11.30	3.70
HCO_3	1.04	12.84	26.63	1.25	21.50	30.62	1.88	28.86	41.09
Cl	20.52	12.75	6.84	19.34	5.63	1.17	18.57	3.67	0.92
SO_4	26.08	18.94	10.19	26.20	8.58	3.97	25.49	5.66	4.29
NO_3	1.60	1.25	0.37	1.54	0.76	0.23	1.40	0.51	0

TABLE 13

Anions dissolved from soil 907 by successive extraction

	RATIO OF SOIL TO WATER								
	1:2			1:5			1:10		
	First extraction	Second extraction	Third extraction	First extraction	Second extraction	Third extraction	First extraction	Second extraction	Third extraction
	Reacting value								
CO_3	0.30	0.93	1.10	2.23	3.13	2.10	5.99	5.99	1.50
HCO_3	4.82	3.82	3.46	7.68	5.36	4.12	10.50	5.25	5.87
Cl	1.16	0	0	1.01	0	0	0.99	0	0
SO_4	5.97	1.52	0.83	5.74	1.18	0.54	6.01	1.85	0.66
NO_3	0.45	0.03	0	0.45	0.05	0	0.47	0	0
	Reacting value in percentage								
CO_3	1.23	7.38	10.20	6.52	16.10	15.53	12.50	22.88	9.34
HCO_3	19.75	30.32	32.10	22.44	27.57	30.48	21.91	20.05	36.55
Cl	4.75	0	0	2.95	0	0	2.07	0	0
SO_4	22.42	12.06	7.70	16.77	6.07	3.99	12.54	7.07	4.11
NO_3	1.84	0.24	0	1.32	0.26	0	0.98	0	0

TABLE 14

Anions dissolved from soil 908 by successive extraction

	RATIO OF SOIL TO WATER									
	1:5				1:10				1:20	
	First extraction	Second extraction	Third extraction	Fourth extraction	First extraction	Second extraction	Third extraction	Fourth extraction	First extraction	Second extraction
	Reacting value									
CO_3	93.91	6.73	4.00	2.00	95.64	12.99	4.00	1.00	93.41	12.49
HCO_3			6.05	4.74		12.76	6.62	4.76		20.01
Cl	222.45	5.55	0.25	0	224.22	3.50	0.25	0	229.52	4.48
SO_4	34.26	1.84	0	0		1.91	0.42	0	35.28	0.75
NO_3	23.94	0.42	0.21	0.14	23.46	0.29	0.18	0.11	23.89	0.11
	Reacting value in percentage									
CO_3			19.03	14.53		20.65	17.44	8.52		16.50
HCO_3			28.78	34.45		20.29	28.86	40.54		26.44
Cl			1.19	0		5.56	1.09	0		5.92
SO_4			0	0		3.07	1.83	0		0.99
NO_3			1.00	1.02		0.46	0.78	0.94		0.15

The percentage of the different anions in a given solution also was calculated, the assumption being made that CO_3, HCO_3, Cl, SO_4 and NO_3 were the only anions present in these solutions. As a matter of fact, small amounts of PO_4 also were present and possibly SiO_3 as well, but the amounts were too small to affect these calculations materially. Since the anions and cations of a solution are numerically equal, the total of either is responsible for only 50 per cent of the total reacting value of the solution.

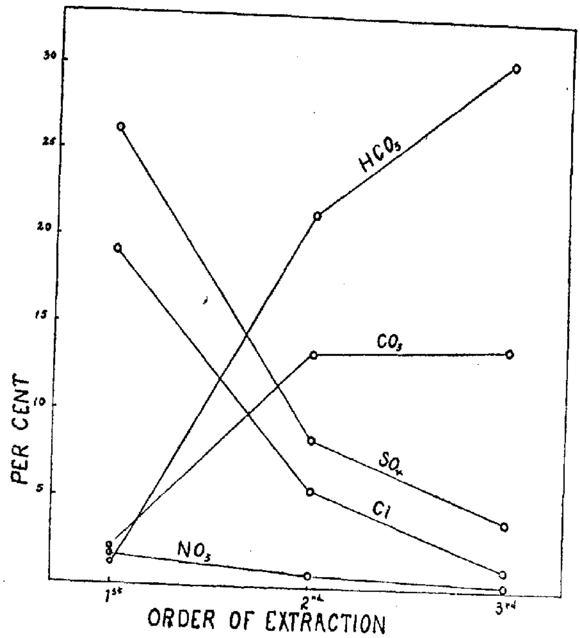

FIG. 11. REACTING VALUE OF THE ANIONS IN SUCCESSIVE EXTRACTS OF SOIL 905 EXPRESSED AS PERCENTAGE OF THE TOTAL REACTING VALUE OF THE SOLUTION

From data showing the reacting value of the different ions, together with their percentages, the true chemical nature of a solution may be readily determined. Such data afford a convenient basis for making strictly chemical comparisons between solutions of different composition and concentration; whereas their true chemical relationships may not be apparent from the ordinary analysis.[4]

[4] The chemists of the United States Geological Survey make extensive use of this principle in studying the composition of solutions (11, 13). As pointed out by Hoagland and Christie (9), agricultural chemists have not adopted it generally. Extensive use of this

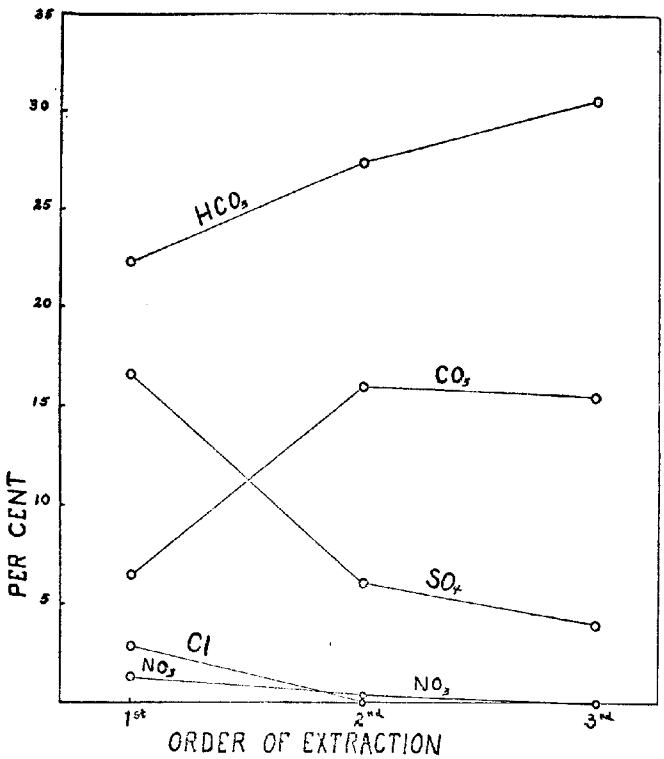

FIG. 12. REACTING VALUE OF THE ANIONS IN SUCCESSIVE EXTRACTS OF SOIL 907 EXPRESSED AS PERCENTAGE OF THE TOTAL REACTING VALUE OF THE SOLUTION

principle is made in this laboratory in studying the composition of soil extracts and irrigation waters.

It is also unfortunate that agricultural chemists should continue to report the numerical amounts of the several salts that are supposed to occur in alkali soils and irrigation water. As a matter of fact, the constituents of solutions exist in the form of ions, either in part or wholly, and not as salts. Furthermore, all of the various schemes in use for combining the different ions in mixed salt solutions are largely hypothetical and belong essentially to the realm of guess work. Moreover, Anderson and Fry (1) have shown recently that when aqueous extracts of soils are evaporated the salts crystallize out, not entirely as the simple salts of commerce, but in part as complex double salts. It would appear to be more logical, therefore, to report the ions actually determined without speculating as to their hypothetical combinations.

It will be noted that the chemical nature of the first extract of each soil was substantially different from that of subsequent extracts. The anions of the first extracts were mainly chloride (soil 908), sulfate (soil 907) or both (soil 905). The second extracts contained relatively greater percentages of carbonate and bicarbonate than the first, and finally the third and fourth extracts were composed very largely of carbonate and bicarbonate, the latter predominating.

Thus it seems quite evident that the relationship between soils and alkaline sodium salts is quite different from that between soils and neutral sodium salts. As will be shown in a subsequent paper from experiments conducted in the open field, repeated flooding accompanied by artificial drainage has thus far failed to reduce the content of sodium carbonate of one of these soils to a point sufficiently low to permit satisfactory growth of barley.

Many investigators have suggested that alkaline salts are adsorbed to a greater degree than neutral salts, but whether adsorption or the formation of chemical compounds of low solubility, as a result of reactions that take place between the alkaline salts and soil silicates or organic matter, is responsible for this fact, cannot now be stated with certainty. It seems probable that the occurrence of compounds of low solubility has more to do with the observed facts than the phenomenon of adsorption.[5] Whatever may be the true explanation of these data, however, they show that it is extremely difficult to remove the last trace of sodium carbonate from soils by leaching.

It seems appropriate to emphasize the fact that the difficulties encountered in removing sodium carbonate from alkali soils by leaching have usually not been recognized sufficiently, either by investigators of the alkali problem, or by those engaged in alkali reclamation. It is safe to conclude that the application of some neutralizing agent in addition to artificial flooding will be helpful in the treatment of any soil that contains black alkali, and that with certain soils the application of a neutralizing agent will probably be absolutely essential to their permanent reclamation. Hilgard proposed the use of gypsum for this purpose. In a subsequent paper we will discuss the use of various other neutralizing agents.

In a previous paper from this laboratory (10) it was shown that non-alkali soils absorb considerably more sodium from solutions of sodium carbonate or sodium hydrate than from solutions of neutral sodium salts. It was also shown that neutral sodium salts react with soils by double decomposition, forming compounds relatively high in sodium but of low solubility. In a subsequent paper it will be shown that the insoluble compounds formed by double decomposition hydrolyze in aqueous suspension yielding strongly alkaline solutions (see a recent paper by Dominicis (5)). It is possible that a portion of the alkalinity of the second and third extracts noted above may have been due to the hydrolysis of such compounds.

[5] See a recent paper by Bouyoucos (2).

SUMMARY

1. The use of compressed air in filtering extracts of alkali soils was not found to affect the content of CO_3 or HCO_3 materially.

2. The amount of total solids dissolved by water was found to increase as the time of shaking was increased, but no consistent variation was found in the amount of any one of the anions. The conclusion is drawn that shaking for 1 hour brings about approximate equilibrium between water and the soils studied.

3. The total amounts of CO_3 and HCO_3 removed from two of the soils studied were found to increase as the ratio of water to soil was increased, while a third soil showed no substantial difference in the amount of CO_3 dissolved.

4. Approximately equal amounts of Cl and NO_3 were dissolved by every ratio of soil to water that was used, while in the case of two of the soils studied, the amount of dissolved SO_4 increased somewhat as the proportion of water was increased.

5. The concentration of OH ions in the extracts of two soils was lowest where the ratio of soil to water was 1:2, and substantially increased with dilution, reaching a maximum with one soil when the ratio was 1:10 and with the other soil when the ratio was 1:40.

6. It is pointed out that the pH value of extracts or suspensions of alkali soils may be substantially higher than that of the soil solution as it occurs in the open field.

7. By extracting the same portion of soil successively with water, it was found that greater amounts of normal carbonate were dissolved by the second extraction than by the first; whereas a very large percentage of the Cl, SO_4 and NO_3 were dissolved by the first extraction.

8. Solutions of substantially different chemical nature were obtained from each soil by extracting them with successive portions of water. The first extracts were composed mainly of chloride, sulfate and nitrate, while the succeeding extracts were composed of increasing percentages of carbonate or bicarbonate.

9. It seems evident that alkaline salts are either adsorbed or held in loose chemical combination by soils to a much greater degree than neutral salts.

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NUTRIENT REQUIREMENTS OF CLOVER AND WHEAT IN SOLUTION CULTURES[1]

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The nutrient requirements of the clover plant and the ratio of phosphate, nitrate and potassium best suited for their growth was studied in aqueous culture solutions, containing calcium acid phosphate ($CaH_4(PO_4)_2.H_2O$), sodium nitrate ($NaNO_3$), and potassium sulfate (K_2SO_4), used singly and in combinations. In this work the solutions were prepared according to the triangular scheme (2), in a similar manner as were the ratio studies made in this laboratory with wheat, reported in a former publication (1).

The details of the methods of experimentation were similar to those described in the earlier work. The red clover seeds (*Trifolium pratense*) were germinated in sand, and put in the culture solutions when about ½ inch high. Ten plants were grown in each culture for 35 days, the container holding 250 cc. of the solutions. The solutions were changed from time to time, and an analysis made to determine the amount of phosphate, nitrate and potash absorbed. The concentration of the salts in the solution was 80 parts per million of P_2O_5,NH_3 and K_2O, there being 66 solutions. Some contained each of the salts singly, some had combinations of two salts and others combinations of three, the ratio of the constituents varying in 10 per cent differences. The triangular plan used in the experiment is familiar, and is readily understood when reference is made to figure 1, or to the paper cited.

In figure 1 the points in the triangle which are numbered represent the composition of the culture solutions. No. 1 represents a solution containing only calcium acid phosphate, no. 56 only potassium sulfate and no. 66 only sodium nitrate. The line of cultures 1 to 56 are mixtures of phosphate and potash, the line no. 56 to 66 mixtures of potash and nitrate, and the line no. 1 to 66 mixtures of phosphate and nitrate. The points in the interior represent solutions containing all three constituents; those near the phosphate end are high in that particular element, likewise those in the potash or nitrogen end are high in the respective constituents mentioned. By reference to the earlier work one can easily understand the composition of the solution from its position in the triangle.

In the studies made with young wheat plants, with the use of the complete triangle set of 66 solutions, it was shown that the better growth occurred

[1] Contribution from the United States Laboratories of Soil-Fertility Investigations.

when all three nutrient elements, phosphate, nitrate and potash, were present in the solution, and the best growth in the mixtures which contained between 10 and 30 per cent of phosphate, between 30 and 60 per cent of nitrate and between 30 and 60 per cent of potash. The growth in the solution containing all three elements was much greater than in solutions containing two elements. The absorption in the solutions was also very striking, the greatest absorption occurring where the greatest growth was obtained. The ratio of the materials absorbed by the wheat and its relation to growth, is interesting in connection with the present studies with clover, and this will be discussed later more in detail.

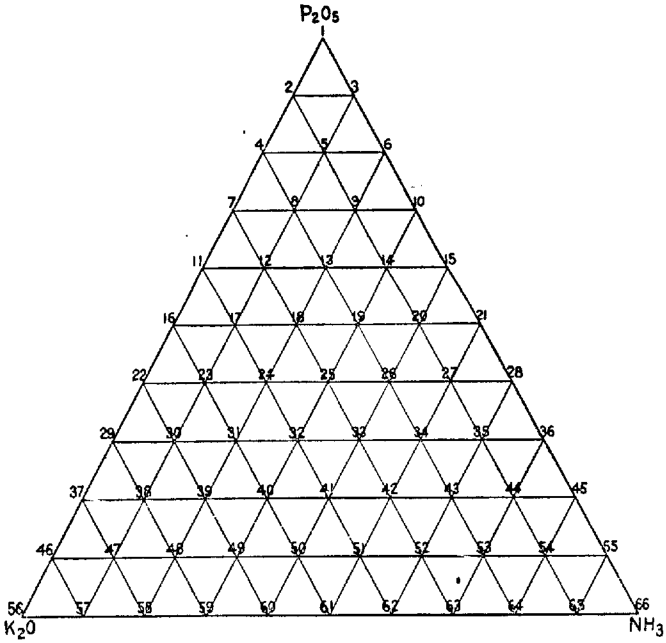

FIG. 1. TRIANGULAR DIAGRAM WITH THE POINTS NUMBERED REPRESENTING THE 66 CULTURE SOLUTIONS

FERTILIZER RATIO AND CLOVER GROWTH

The green weights of the clover plants, which were grown in the 66 solutions from February 25 to March 27, 1918, are given in the chart in figure 2. The complete triangle set as grown in the greenhouse is shown in plate 1, figure 1. The concentration of the solutions was 80 parts per million of P_2O_5, NH_3 and K_2O. The solutions were changed every 5 days, seven changes being made during the experiment, and an analysis made to determine the amount and

ratio of phosphate, nitrate and potash absorbed. It is observed that the largest growth occurs in those solutions in the interior of the triangle, lying somewhat below the center of the figure, a result very similar to that secured with wheat. That is, the growth of both clover and wheat is best in those solutions containing approximately an equal ratio of nitrogen and potash with a relatively smaller proportion of phosphate. On closer examination of the data here given for clover and those for wheat in figure 3, page 10, of the article cited (1), it is seen, however, that the cultures producing the

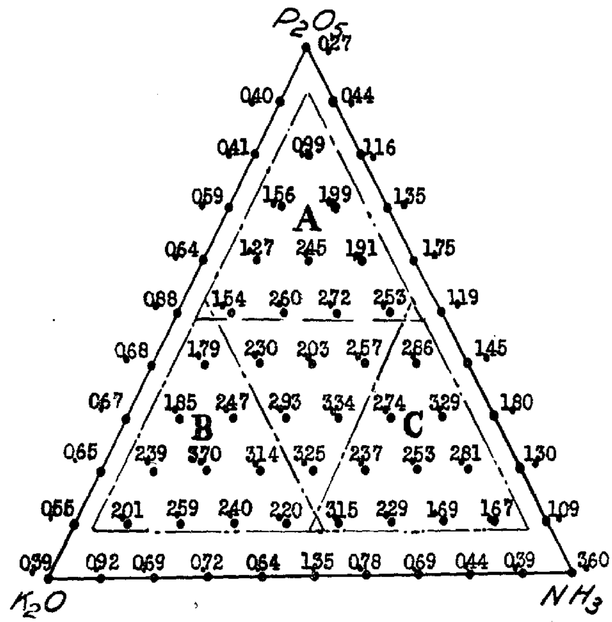

FIG. 2. GREEN WEIGHT OF CLOVER IN GRAMS GROWN IN THE 66 SOLUTIONS

Sub-triangle *A* contains the solutions which have the three salts, but containing principally phosphate; sub-triangle *B* those containing principally potash, and sub-triangle *C* those containing principally nitrogen.

maximum clover growth contained a slightly higher proportion of potash and a slightly lower proportion of nitrogen than the cultures which produced the maximum growth of wheat.

The relation of the ratio of the three nutrients absorbed from the solution by clover and by wheat also is of interest. The data show that the clover absorbs a slightly higher ratio of potash than does wheat, the difference being naturally more marked in those solutions containing a higher proportion of potash and nitrogen than phosphate.

The solution cultures as grouped in figure 2, bring together those containing three salts, and having 50 per cent or more of a single constituent. Sub-triangle *A* contains the solution cultures having a high proportion of phosphate; sub-triangle *B* those having a high proportion of potash and sub-triangle *C* those having a high proportion of nitrogen. The ten cultures of sub-triangles *A*, *B* and *C* produced a total growth respectively of 15.5 gm., 25.4 gm. and 24.5 gm. The larger growth occurred in the high potash and the high nitrogen solutions, the growth in the high potash being slightly greater. The culture (no. 39) which produced the greatest growth, 3.70 gm., contained the nutrients P_2O_5,NH_3 and K_2O in the ratio of 20–20–60. The solution which produced the maximum growth of wheat in the experiment previously cited contained the phosphate, nitrate and potash in the ratio of 20–40–40, the composition of which is higher in nitrogen and lower in potash than the solution which produced the maximum growth of clover.

In plate 1, figure 2 are shown four cultures, one representing each of the sub-triangles just discussed and one from the interior triangle. *A* is a high-phosphate culture and contains phosphate, nitrate and potash in the ratio of 60–20–20; *B* is a high-potash culture and contains the constituents in the ratio of 20–20–60; *C* is a high-nitrogen culture and contains the constituents in the ratio of 20–60–20; *D* has nearly equal proportions of the three constituents, the ratio being 30–40–30. It is seen here that culture *B*, plate 1, figure 2, the high-potash solution, has made a larger growth than the others. The green weights of these cultures, as seen in figure 2, namely solutions 13, 39, 43 and 33, was for *A*, 2.45 gm.; *B*, 3.70 gm.; *C*, 2.53 gm.; and *D*, 3.34 gm. These are representative solutions from each sub-group of the triangle, and it is seen that the solution slightly higher in potash has produced the best growth.

An analysis of the green-weight data shown in figure 2, comparing the cultures containing two constituents with those containing three constituents, is interesting. It is readily observed that in each case the growth where any two constituents are present is less than where three constituents are present, regardless of whether the missing fertilizer constituent is phosphate, nitrate or potash.

Considering first the group of cultures having no phosphate, and 10 per cent phosphate, it is seen that the cultures having no phosphate, that is those along the line 56 to 66 (fig. 1), are much smaller than those having 10 per cent phosphate, as shown in the line 46 to 55. The average green weight of the eleven cultures from the no-phosphate line is 0.641 gm., and that of the cultures from the group of solutions containing 10 per cent of P_2O_5 in its composition is 1.962 gm. There is a decrease of 67 per cent in those cultures where the P_2O_5 was absent from the solution.

In making a similar comparison from the point of view of the absence of nitrate from a solution, the relation between the line of cultures 1 to 56 and 3 to 57 (fig. 1 and 2) should be studied. The average green weight in the no-nitrate solutions, the group of 11 cultures 1 to 56, is 0.536 gm. against

1.474 gm. as the average growth for the group of 10 cultures having 10 per cent of nitrate in their composition. It is here seen that there is a reduction in growth of 64 per cent when the nitrate is absent.

When a similar comparison is made to show the effect of the absence of potash, it is seen that there is not so marked a reduction as when phosphate or nitrate was left out of the solution. The average growth of the line of 11 cultures having no potash, 1 to 66, is 1.105 gm., as compared with 1.882 gm. for the line of cultures, 2 to 65, having 10 per cent of potash. There is a reduction here of only 40 per cent.

From this comparison one might conclude that potash was less important in the metabolism of the clover plant than is phosphate or nitrate. But such is shown not to be the case when a study is made of the complete set of cultures. Clover does not function properly in a solution except in the presence of phosphate, potash and nitrate. It is true that in the experiment, a larger growth was made in solutions containing nitrogen and phosphate than in solutions of phosphate and potash or nitrogen and potash, but this growth, at the best, was poor and far from normal. The larger growth is made in the solutions having all the fertilizer constituents and the maximum growth in the solutions containing a higher ratio of potash than of phosphate or nitrogen. The influence of potash in the growth and metabolism of the clover plant is further considered in the next section of this paper, where it is shown that potash is absorbed in larger proportions than phosphate or nitrogen.

GROWTH AND ABSORPTION

The absorption of the nutrients by both clover and wheat was studied, by analyzing the solutions at the end of each change. The colorimetric methods for determining phosphates, nitrates and potash were used (1). The relation between growth and absorption of the two plants is interesting. The culture jars, arranged in a triangle, in which the plants are growing, and the bottles for storing the solutions for analytical work, are shown in plate 1, figure 3.

These comparative data are given in table 1, which shows the ratio of phosphate, nitrate and potash absorbed from the solution. In the second column is given the original ratio of the solution and in the last column the ratio absorbed by each kind of plant. The solutions which contained only one or two salts are not included in the table; those containing the three salts are given, as these only are of interest in the complete nutrient absorption studies.

The comparison which it is intended should be made in this table is that between the ratio of phosphate, nitrate and potash absorbed by clover, and that absorbed by wheat, from the solution having a similar original ratio. It is seen that in a solution having 50 per cent or more of P_2O_5 in its composition (no. 5 to 20) the ratio of potash absorbed by wheat is in general higher than

TABLE 1

The ratio of phosphate, nitrate and potash absorbed by clover and wheat plants from nutrient solutions containing calcium acid phosphate, sodium nitrate and potassium sulfate—original concentration 80 p.p.m. of $P_2O_5 + NH_3 + K_2O$

SOLUTION NUMBER	ORIGINAL RATIO P_2O_5–NH_3–K_2O	CROP	RATIO ABSORBED P_2O_5–NH_3–K_2O	SOLUTION NUMBER	ORIGINAL RATIO P_2O_5–NH_3–K_2O	CROP	RATIO ABSORBED P_2O_5–NH_3–K_2O
5	80–10–10	Clover Wheat	42–36–22 46–33–21	27	40–50–10	Clover Wheat	33–53–14 29–56–15
8	70–10–20	Clover Wheat	34–30–36 35–24–41	30	30–10–60	Clover Wheat	20–19–61 17–19–64
9	70–20–10	Clover Wheat	50–42–8 35–49–16	31	30–20–50	Clover Wheat	22–31–47 21–34–45
12	60–10–30	Clover Wheat	31–31–38 28–19–53	32	30–30–40	Clover Wheat	22–29–49 19–38–43
13	60–20–20	Clover Wheat	41–37–22 32–36–32	33	30–40–30	Clover Wheat	21–47–32 21–42–37
14	60–30–10	Clover Wheat	45–40–15 32–51–17	34	30–50–20	Clover Wheat	19–50–31 17–58–25
17	50–10–40	Clover Wheat	26–21–53 24–18–58	35	30–60–10	Clover Wheat	28–59–13 23–63–14
18	50–20–30	Clover Wheat	31–33–36 27–33–40	38	20–10–70	Clover Wheat	17–19–64 16–21–63
19	50–30–20	Clover Wheat	31–39–30 28–41–31	39	20–20–60	Clover Wheat	17–27–56 16–37–47
20	50–40–10	Clover Wheat	32–42–26 33–51–16	40	20–30–50	Clover Wheat	16–37–47 15–40–45
23	40–10–50	Clover Wheat	36–23–41 25–21–54	41	20–40–40	Clover Wheat	13–42–45 15–41–44
24	40–20–40	Clover Wheat	27–27–46 24–32–44	42	20–50–30	Clover Wheat	16–44–40 16–48–36
25	40–30–30	Clover Wheat	27–33–40 22–40–38	43	20–60–20	Clover Wheat	16–51–43 18–55–27
26	40–40–20	Clover Wheat	28–36–36 26–45–29	44	20–70–10	Clover Wheat	20–65–15 21–66–13
47	10–10–80	Clover Wheat	13–20–67 11–18–71	51	10–50–40	Clover Wheat	11–46–43 12–46–42

TABLE 1—*Continued*

SOLUTION NUMBER	ORIGINAL RATIO $P_2O_5-NH_3-K_2O$	CROP	RATIO ABSORBED $P_2O_5-NH_3-K_2O$	SOLUTION NUMBER	ORIGINAL RATIO $P_2O_5-NH_3-K_2O$	CROP	RATIO ABSORBED $P_2O_5-NH_3-K_2O$
48	10-20-70	Clover	10-28-62	52	10-60-30	Clover	10-57-33
		Wheat	13-38-49			Wheat	12-52-36
49	10-30-60	Clover	8-33-59	53	10-70-20	Clover	9-61-30
		Wheat	13-42-45			Wheat	13-60-27
50	10-40-50	Clover	10-38-52	54	10-80-10	Clover	14-66-20
		Wheat	11-42-47			Wheat	17-70-13

that absorbed by clover. This group of solutions contains a relatively small proportion of nitrogen and potash but is high in phosphate. In the other solutions, lower in phosphate but higher in either nitrate or potash, the ratio of the constituents absorbed is quite different. The ratio of potash absorbed by clover was greater than with wheat in 19 solutions out of the group of twenty-six. From these culture experiments it becomes apparent that the clover absorbs a smaller proportion of nitrogen and a larger proportion of potash than does wheat.

These data are probably more easily grasped by an examination of figure 3, where the results given in table 1 are shown diagrammatically. The original ratios of these 36 solutions are represented by the origin or junction of the solid and broken line, the location on the diagram corresponding to the scheme previously explained and given in figure 1. The large black dot represents the ratio of the nutrients absorbed from that solution by the clover, and the small circle the ratio of the nutrients absorbed by wheat. The diagram deals only with the ratios of the ingredients and not with the amounts that are absorbed. The dot is connected to its original solution by a solid line and the circle by a broken line. Thus it is possible to see at a glance the ratio of the materials absorbed by the two kinds of plants from each solution.

That the diagram may be fully understood, let us discuss the solution represented by the point at the junction of the lines in the lower right-hand corner of the diagram. This solution is no. 54, as shown in figure 1. From table 1 it is seen that the original ratio of P_2O_5, NH_3 and K_2O of this solution is 10-80-10. This is also apparent from its location on this diagram. As seen in the table the clover absorbed the P_2O_5,NH_3 and K_2O in the ratio of 14-66-20. This ratio is located on the diagram (fig. 3) at the black dot which is connected with its original solution by the solid line. The wheat absorbed the three constituents from the original solution in the proportion of 17-70-13, which is located on the diagram at the circle and is connected to the original solution by the broken line. The clover absorbed a higher proportion of potash than did wheat, and the wheat a higher proportion of

nitrogen than did clover. This is shown in the figure by the fact that the dot lies nearer the potash end of the triangle than does the circle. With the exception of the solutions in the high-phosphate, or upper part of the triangle, it is apparent that in most of the solutions the dots lie nearer to the potash end of the triangle than do the circles. This is especially true of the solutions near the center of the triangle which originally contained the potash and nitrate in nearly equal proportions.

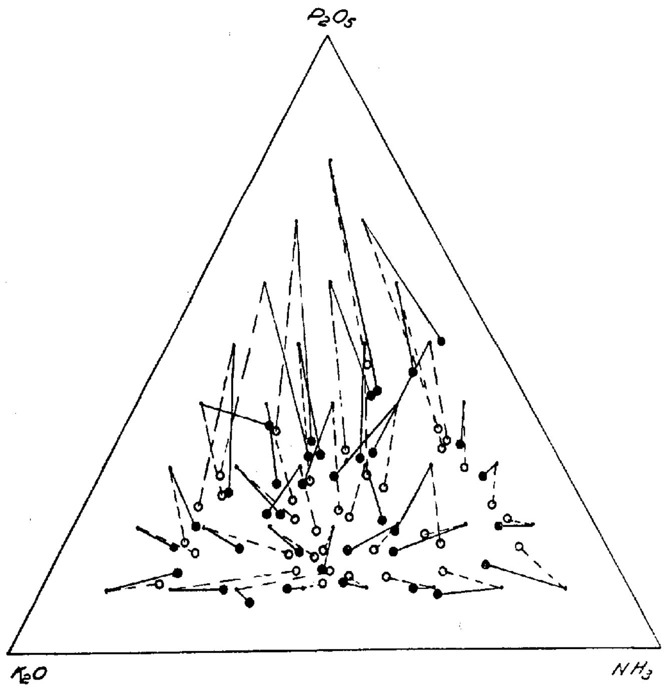

FIG. 3. DIAGRAM SHOWING RATIO OF P_2O_5, NH_3 AND K_2O ABSORBED BY CLOVER AND BY WHEAT FROM THE NUTRIENT SOLUTIONS OF THE TRIANGLE SET

The points at the junction of the solid and broken line represent the ratio of phosphate, nitrate and potash of the original solution (fig. 1). The circles represent the ratio of the three fertilizing constituents absorbed by wheat, and the black dots the ratio absorbed by clover. The circle is connected to its original solution by a broken line and the dot by a solid line.

Note that in any pair the black dots showing absorption by clover are generally on the left or nearer to the potash angle of the triangle, and the circle showing the absorption by wheat is generally on the right, or nearer the nitrate angle of the triangle.

It would seem that the ratio of absorption of the fertilizing constituents, P_2O_5,NH_3 and K_2O by clover and wheat are somewhat different, The clover requires a higher proportion of potash, and a smaller proportion of nitrogen than the wheat. The results also indicate that a higher ratio of potash, in a solution containing all three fertilizing constituents, is required for the maximum growth of clover than for the maximum growth of wheat. Conversely, the wheat absorbs and requires for its maximum growth a higher ratio of nitrogen than does clover.

In table 2 the four solutions producing the maximum growth of clover, as recorded in the diagram of figure 2, are given, together with the original ratio of P_2O_5,NH_3 and K_2O and the ratio of these constituents absorbed. There are also given the four solutions producing the maximum growth of wheat, as recorded in figure 3, page 10, of the article cited (1). Here, too, the original ratio of the constituents and the ratio absorbed by the wheat are presented.

TABLE 2

The original ratio of P_2O_5, NH_3 and K_2O and the ratio of these nutrients absorbed by clover and by wheat from the four solutions of each set producing the largest growth

CLOVER				WHEAT			
Solution number	Original ratio: $P_2O_5-NH_3-K_2O$	Ratio absorbed: $P_2O_5-NH_3-K_2O$	Green weight	Solution number	Original ratio $P_2O_5-NH_3-K_2O$	Ratio absorbed: $P_2O_5-NH_3-K_2O$	Green weight
			gm.				*gm.*
39	20–20–60	17–27–56	3.70	33	30–40–30	21–42–37	4.96
40	20–30–50	16–37–47	3.14	41	20–40–40	15–41–44	5.24
41	20–40–40	13–42–45	3.25	50	10–40–50	11–42–47	5.06
51	10–50–40	11–46–43	3.15	51	10–50–40	12–46–42	5.16
Average...	17–35–48	14–38–48		Average..	17–43–40	15–43–42	

The solutions producing the maximum growth of clover contained the constituents, P_2O_5, NH_3 and K_2O in the ratio of 20–20–60 (no. 39), while the solution producing the maximum growth of wheat contained the three fertilizing constituents in the ratio of 20–40–40 (no. 41). The four solutions from the triangle set producing the largest growth of clover, as seen in table 2, contained the constituents in an average ratio of 17–35–48, while the four solutions producing the greatest growth of wheat contained the fertilizing constituents in an average ratio of 17–43–40. Here it is seen that clover requires a higher ratio of potash than of phosphate or nitrogen for its maximum growth, and wheat a higher ratio of nitrogen than of phosphate or potash.

The absorption of the three fertilizing constituents also is interesting. The clover in the four solutions producing the greatest growth absorbed the phosphate, nitrogen and potash in the ratio of 14–38–48, and the wheat absorbed these constituents from its four solutions in the ratio of 15–43–42. The relative absorption of phosphate, nitrate and potash by clover was greater

for potash and the absorption of wheat was somewhat greater for nitrogen. This result is shown not only by the four solutions producing maximum growth just discussed, but is also revealed generally by the data given in figure 3.

The results seem in harmony with the generally accepted idea that clover is a heavy potash-feeding plant and is also in harmony with results secured by the senior author in a field experiment where the triangle fertilizer scheme was used (3). This fertilizer experiment in which acid phosphate, sodium nitrate and potassium chloride were used, was made on Hagerstown loam soil and has been conducted for about 10 years. The composition of the vegetation was originally Canada blue-grass, Kentucky blue-grass, timothy and white and red clover. At the end of 7 years the differently fertilized plots contained the various species in distinctly different proportions. Grass generally has predominated over clover in the plots receiving fertilizers with high ratios of nitrogen, while clover, especially red clover, has been crowded out of such fertilized plots. Clover existed in the struggle more easily in the plots fertilized with mixtures of potash and phosphate, being much more abundant in that section of the triangle.

Therefore, similar conclusions can be drawn from the two experiments, one in aqueous solutions, the other in field plots. In a general way both point to the conclusion that the clover plant requires a higher proportion of potash than of nitrogen or of phosphate, in its metabolism. It should be remembered, however, that the fertilizer requirements of any particular soil will upset this ratio requirement of the plants; the best fertilizer ratio for clover production will vary with different soils. The poor clover soils of the Volusia and Scottsburg series of southern Indiana are most improved for clover production by lime, manure and complete fertilizers. Nitrogen and phosphate appear to be more important than potash. On the other hand, the potato soils of the Cape Charles section of Virginia and of Maine where large amounts of high-potash carrying fertilizers have been used for years, produce clover luxuriantly, undoubtedly in part because of the high potash fertilization.

The results point to the conclusion that normally clover is a potash-loving plant. The true nutrition requirement of the clover plant can be derived from aqueous culture solutions where many factors are controlled. The fertilizer requirements of soils for clover production, where many soil factors play a part, is an entirely different matter. Each soil type, under each soil condition, may have a different fertilizer requirement, making it necessary to study each soil problem separately.

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PLATE

PLATE 1

FIG. 1. Clover in triangle set of nutrient solutions, showing equipment used in the experiment and the greenhouse in which the plants grew.

FIG. 2. Clover grown in nutrient solutions of calcium acid phosphate, sodium nitrate and potassium sulfate and having varying ratios of P_2O_5, NH_3 and K_2O.

A, Culture 13 of triangle set, having P_2O_5, NH_3 and K_2O in ratio of 60–20–20

B, Culture 39 of triangle set, having P_2O_5, NH_3 and K_2O in ratio of 20–20–60

C, Culture 43 of triangle set, having P_2O_5, NH_3 and K_2O in ratio of 20–60–20

D, Culture 30 of triangle set, having P_2O_5, NH_3 and K_2O in ratio of 30–40–30

FIG. 3. Clover in nutrient solution triangle set, showing bottles for keeping solutions for analytical work in the center case, new solutions in the culture jars on the right and the clover cultures growing on the left.

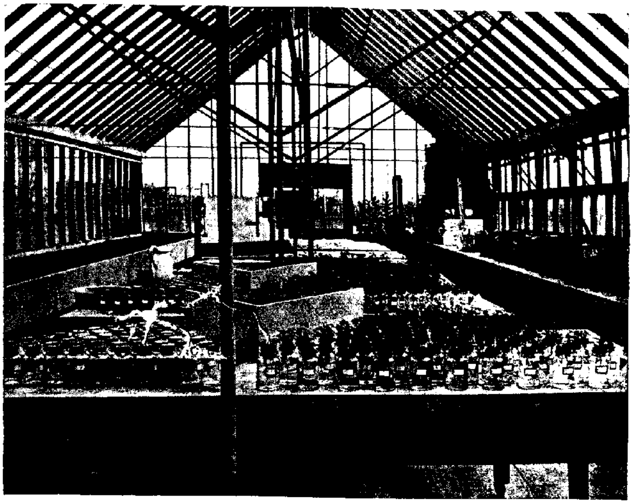

Fig. 1

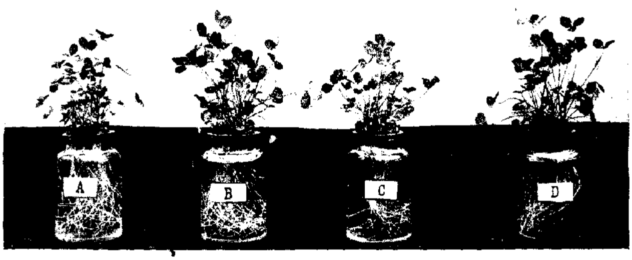

Fig. 2

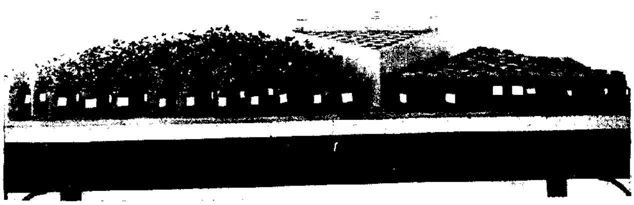

Fig. 3

NITRIFICATION IN SOME SOUTH AFRICAN SOILS

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INTRODUCTION

Although the study of the rate and amount of nitrate formation, as a means of judging the biological activity and also the general fertility of the soil, has received a great deal of attention in Europe and America and also some attention in India, Australia and other countries, the writer can find no record of nitrification in soil having received any similar quantitative attention by the agricultural chemists of this country. Juritz (16, 17, 18), Ingle (47), Watt (47, 48), Vipond (47), Marchand (32), and others have all contributed, some very largely, to the amount of data which have been accumulated on the chemical composition of South African soils.

Juritz (16) has published investigations on 40 per cent more soils than all the above mentioned workers together, and using the standard of Maercker as his criterion shows that 52 per cent of the soils of the Cape Province are deficient in nitrogen. Marchand (32) has recently summarized the data accumulated by the investigators of Transvaal soils, and concludes that if a deficiency in nitrogen is assumed, when a soil contains less than 0.1 per cent, then 55 per cent of the soils of this province are deficient in this element. The standards of Maercker and Dyer as used by Juritz and Marchand, are those obtaining in German and English climatic conditions, which are very different from those of South Africa. It has often been observed that the application of mineral nitrogenous fertilizers to soils in South Africa, which are below the European standards in nitrogen content, have not given the expected returns from the fertilizer, and in some cases no returns at all. The only manner of applying nitrogen that has given a monetary return on the soils of this experiment station, has been with legumes as a green manure. Consequently, this station does not recommend to farmers the practice of applying mineral nitrogenous fertilizers except in rare cases. The fact cannot be overlooked that the poverty of the average South African soil in phosphates may be quite sufficient to account for the lack of response of the soil to mineral nitrogenous fertilizers applied alone.

Soils even poor in nitrogen according to European standards often give excellent returns when the rainfall has been sufficient and no nitrogenous fertilizer has been applied.

Hilgard (14) long ago found that Maercker's standard was too high under California conditions.

The writer concurs with Marchand in his opinion that although not sufficient data are at present available for a definite lower standard under these conditions, an arbitrary one might be tentatively adopted.

Marchand gives as his reason that nitrification is very rapid in South African soils, and therefore, a lower nitrogen standard would be advisable. Opinions in general on the intensity of nitrification in semi-arid soils have in the past been based largely on Hilgard's statements of his observations on Californian soils, and the chemists of South Africa have preached that doctrine chiefly on account of the similarity of our climate to that of western America. Later workers in that area have greatly modified the views held by Hilgard, and some differ wholly from them.

Some of the work of the chief investigators of the nitrification problem in western American will be reviewed later on.

THE OBJECT OF THIS INVESTIGATION

It was with the object of getting some quantitative data on nitrification in South African soils and so a basis for comparing our soils with those of other continents, and ascertaining whether the process of nitrate formation is as active as has been assumed, that the writer undertook the investigations here recorded.

REVIEW OF SOME PREVIOUS WORK

Watt, as far as the writer can ascertain, is the only investigator who has attempted this problem in South Africa. His work is recorded in the Annual Report of the Transvaal Department of Agriculture, 1907–08. His studies were of a qualitative nature in so far as it has not enlightened us on the amounts of nitrate nitrogen occurring in our soils, but was quantitative in that it gives the time taken for 0.4 gm. of soil to transform the nitrogen, in the modified Winogradsky's solution as used by Hall at Rothamsted, into ammonia nitrite and nitrate. Watt found that nitrification in this solution was complete in 28 to 46 days, and that the two soils that were quickest in this respect contained 1.46 and 1.56 per cent of lime, and 0.141 and 0.174 per cent of nitrogen, respectively. The nitrification of this solution with English soils under similar conditions, he states, was often not complete in 60 days. If then, he concludes, Transvaal soils are so much superior in nitrifying power to English soils under similar conditions, how much more superior must they be during the actual summer months of good rainfall, when the day and night temperatures are considerably higher than those in England? He found that even poor, sandy soils, containing only a little organic matter and a trace of lime nitrified the above solution more quickly than the best English soils. Watt further states, "This would be the explanation of the fact that in the Transvaal

certain soils that would be considered too poor to cultivate in temperate climates, are capable of producing good crops without nitrogenous manuring."

The writer has not corroborated Watt's results with a similar method, as during the last 8 years most soil biologists and chemists have adopted the method of studying the amount of nitrate produced in the soil itself, either from its own nitrogen or together with added nitrogen, and similar methods to these have been used by him, so as to make the results comparable with recent work. Löhnis and Green (26), however, have shown very clearly that when the proper precautions are exercised the solution method gives quite as good an indication of the nitrifying power of the soil as the soil method.

Most of the work on nitrification on semi-arid soils has been done in western America. Hilgard noticed the high amount of nitrate in the alkali soils of California, and attributed it to intense nitrification.

Stewart (43), however, points out that the same causes that accumulated the other water-soluble salts probably accumulated the nitrates, and gives instances of observations of high amounts of nitrates recorded in Turkestan, India and China, but always associated with correspondingly high amounts of other water-soluble salts, while Hilgard attributed the high amount of nitrate to the nitrification of organic matter taking place at the present time. Headden (12, 13) concludes that the high percentage of nitrate in the brown spots on arable land in Colorado is due to the fixation of atmospheric nitrogen *in situ* and the subsequent nitrification of the dead bacterial flora. These processes, he says, are going on actively today.

It is a significant fact, though, that Headden's analyses of the water-soluble salts of the soils studied, show high percentages of chlorides and sulfates. Still, he determined that large amounts of nitrogen are fixed, if soils from some of these nitre spots are left in the laboratory at a suitable moisture content.

The amount of nitrogen fixed according to Headden and Sackett (39) amounts in some cases to thousands of pounds per acre.

Stewart (43) in Utah, with climatic and soil conditions similar to those of Colorado, found that nitrification added only 28 pounds of nitric nitrogen per acre per annum, and he quotes Warington as giving 86.5 to 89.5 pounds per acre as the amount formed under humid conditions at Rothamsted, while the highest amount the writer has observed in cultivated fallow non-irrigated land at Potchefstroom in a year, has been 84.9 pounds per acre foot. At no time on the irrigated plots did he find as much as this, while the nitrate found on alkali irrigated soil at Klerksdorp amounted to only 23.6 pounds per acre foot, with the total water-soluble salts per acre foot as 13,200 pounds. Stewart also found in the shale cliffs from which some of the soils of both Colorado and Utah are formed 66 to 354 parts per million of nitrate, and in some places 2000 parts per million (43, 44). This he states is sufficient to account for the high nitrate content of the soils. Shales in South Africa around Prieska and Hay have been found to contain potassium nitrate in large quantities, 0.06 to 9.97 per cent; the origin is doubtful but it is thought that it may have been

derived from the nitrification of the excrements of birds and small animals, and have accumulated through centuries, the rainfall of the area being low (9).

Stewart (42) found in other parts of Utah only small quantities of nitrate in soils not containing other water-soluble salts, many having less than 1 part per million of nitric nitrogen, the highest containing only 4.5 parts per million, and most having not more than 2.4 parts per million. This is strongly in accordance with the amounts of nitric nitrogen found in field samples of various South African soils by the present writer. The writer in the summer of 1913 spent considerable time on several of the ranches from which Stewart took samples, and found the nature of the soil, the method of cultivation and fallowing under 18 inches of rainfall such as to encourage maximum nitrification. Also, the crops of wheat and barley produced were far above our averages in South Africa.

Stewart and Greaves (44) also worked on irrigated Utah soil, ideal for nitrigen fixation and nitrification and giving strong cultures of *Azotobacter;* they did not get anything like the increases in nitrates that are reported by Headden. They point out that in a great many cases where Headden records increases in nitrates, his own figures show increases in chlorides as well. They do not deny that there may be very intense nitrogen fixation in Colorado soil, but from Headden's own results they have correlated the nitrogen and chlorine and say that any explanation that accounts for increases in nitrates must also account for increases in chlorides, and on that account the explanation of fixation from the atmosphere will not suffice.

Sackett (39), however, shows that the rate of the fixation of nitrogen by soils from these brown nitre patches is sufficient to account for all the nitrates found, *if all the nitrogen that is fixed is nitrified*. Again in 1914 he maintains that the nitrates are formed *in situ* and cannot be accounted for by the amount of nitrate in the irrigation water which is only 0.1 to 0.6 part per million. Neither does the drainage water from soils with rich nitre patches contain more than a trace of nitrate, so Sackett concludes the nitrates are not accumulated from water by surface evaporation (40). He has also shown in his second mentioned work that Colorado soils have excellent nitrifying powers, and in comparing them in this respect with soils from other sections of the United States found the Colorado soils to be much superior in the nitrifying of dried blood, ammonium chloride, carbonate and sulfate. Even then he does not think that this active nitrification could account for the excessive accumulations of nitrate, as Colorado soils are deficient in organic matter. Hutchinson (15) shows very clearly that nitrogen fixation can be increased by adding nitrogen-free organic matter to the soil, and thus it would seem that the lack of organic matter in Colorado soils might also be a factor against excessive fixation.

Headden in 1918 says that he has now too many instances at his disposal to entertain the idea that the high nitrate content of the shales is sufficient to explain the nitre spots in Colorado soils; some of these spots occur hundreds

of feet above the shale, others below it and others nowhere near shale beds (13). He also states that nearly all the irrigation waters are very low in nitrate content, and is still firmly convinced that the nitrogen gets into the soil by direct fixation in these nitre spots which contain more active bacteria than have been hitherto found in other parts.

The only instance of which the writer knows of an exceptionally high nitrate spot in a South African soil is that mentioned by Juritz in the Colesburg area, which contained 0.3 per cent of total water-soluble salts including 0.105 per cent of calcium nitrate and 0.086 per cent of magnesium nitrates (17).

Buckman (5) working on non-irrigated land in Montana, found in cultivated fallow nitrates up to 66.5 parts per million. Scott and Robertson (36) in Australia report nitric nitrogen at the rate of 24 parts per million in fallow land.

Recently Prescott (35) in Egypt reports 35 parts per million of nitric nitrogen in fallow land, but on the ridges of irrigated land he found 601 to 697 parts per million. This, however, was always accompanied by large quantities of other soluble salts. Tulaikoff (46) in a section of semi-arid Russia did not find more than 22 parts per million of nitric nitrogen in fallow soil, as a 5-year average.

The workers on Colorado soils are the only ones of whom the writer is aware who report such excessive quantities of nitrate in soil, which nitrate is not associated with quantities of other water-soluble salts, or heavily impregnated irrigation or seepage waters.

By far the most work that has been done on comparing the nitrifying powers of humid and arid soils is that by Lipman, Burgess and Klein (24). These investigators are of opinion that the nitrifying powers of arid soils are no more intense than those of humid regions, while they admit their data are not quite positive, the conclusion seems possible that the humid soils are better. If the efficiency of the two lots of soils is compared only on the percentage of the soil nitrogen oxidized, this conclusion can be drawn.

It would seem that the amounts of nitrate recorded by workers on arable soils in semi-arid regions in general are low, when compared with high amounts found in the humid region of Ithaca, New York, by Lyon and Bizzell (28). In making this statement the writer excepts the high amounts found in nitre spots, and these amounts associated with quantities of other water-soluble salts on alkali soils. Lyon and Bizzell found in their seasonal variation studies on bare soil 180 parts per million frequently and often over 200 parts per million.

On semi-arid lands the highest figure of which the writer knows, is that of Buckman in Montana, with 66 parts per million and the writer found in midsummer in Potchefstroom, 55 parts per million.

OUTLINE AND SCOPE OF THIS WORK

Before further discussing the problem the writer will record the results of his own investigations, which bear also on other points besides those of inten-

sity of nitrification. In broad outline the purpose and scheme of the work was to study and obtain data on some of the following phases of nitrification under South African conditions:

1. To ascertain the amount of nitric nitrogen in the soil around Potchefstroom, virgin, cropped and cultivated at various seasons of the year and after various crops.
2. To correlate the seasonal variations of nitrates with our climatic conditions.
3. To ascertain the amount of nitric nitrogen in the first 5 feet of the soil in various sections of this locality.
4. To compare the nitrifying powers of each of the 5 feet, and see how far nitrification is active.
5. To see how much limestone and slaked lime affected nitrification on dry-land plots growing potatoes.
6. To compare the nitrifying powers of our irrigated and long cultivated land with virgin dry lands, in regard to various amounts of blood-meal and ground limestone applied.
7. To find out how much nitric nitrogen was added to a soil by cowpeas alone, lime alone, and by mixtures of the two.
8. To compare the nitrifying powers of soils from diverse parts of the Union with one another, and to ascertain the nitrogenous fertilizer most easily nitrifiable.
9. To compare the nitrifying powers of virgin and cultivated soils of the same type.
10. To correlate, if possible, the nitrifying power of soils with the amount of rainfall in the area from which they came, and also with the organic matter, total nitrogen content, and lime requirement.
11. To compare the nitrifiability of whale guano variously treated.
12. To compare the nitrifiability of small and large amounts of nitrogenous fertilizers, and to ascertain if possible which is better for comparing the nitrifying powers of various soils.

EXPERIMENTAL PROCEDURE

As the writer had had considerable experience in the manipulation of the phenol-disulfonic acid colorimetric method of determining nitrates, while working in the soil technology laboratories of Cornell University, this method was selected for use in these investigations. The method is very quick and also accurate. It is described in Bulletin 31 of the Bureau of Soils (41). It is endorsed by Lipman and Sharp (25), Kelly (19) and Noyes (34), and is used by Lyon and Bizzell (30) and many other workers in this field.

Kelly compared this method with that of the aluminum-reduction method as outlined by Burgess (6), and found that it was in every respect as accurate and in some cases more accurate.

The method used at first was as outlined in Bulletin 31, but later on the Pasteur-Chamberland filter tube got broken, and as another could not be secured the Noyes modification was adopted. Both Noyes, and Lipman and Sharp show in their papers that clarifying the soil solution with slaked lime before filtering does not interfere at all with the accuracy of the nitrate determination, and the writer finds this method considerably quicker than the filter-tube one, unless a whole battery of filter tubes are at one's disposal.

METHOD OF SAMPLING IN FIELD

The soil at this station becomes so hard and dry during a large part of the year, that it is very unsatisfactory and in fact practically impossible to use a soil auger. A pick and spade were used in taking all the samples. Four holes were dug diagonally across a $\frac{1}{20}$-acre plot, each 1 foot deep. A vertical slice of soil about $1\frac{1}{2}$ inches wide was cut off from the side of the hole and placed on a clean rolling cloth, the four slices were passed through a $\frac{1}{4}$-inch mesh sieve and thoroughly mixed by rolling, and a representative sample placed in a glass screw-top fruit jar, and conveyed immediately to the laboratory, where the moisture content and the nitrate content were determined.

I. PRELIMINARY STUDIES

A number of preliminary studies are recorded in table 1. These determinations were made to obtain some idea of the amount of nitric nitrogen, in the soil on various portions of the farm at this station before the experiments proper were started. This was early in June, 1919, and the cold weather had already set in. Soil from a dry-land virgin plot—from two separate samples taken on June 1, gave nitrates in small quantity but in very close agreement. During this cold month the nitrates had actually increased from 0.48 to 2.9 parts per million of nitric nitrogen, as will be seen by the determinations made on June 1 and 30 from the same spot.

The soil from the cultivated irrigated soil on June 30 contained only 0.44 parts per million of nitric nitrogen while the soil on the dry-land cultivated plots of the same type as the dry-land virgin had 38 parts per million on June 10 and 18 parts per million on July 11, 1919. This shows the benefits from cultivation and the increased water-holding capacity of the soil on nitrate production. The nitrates in the cultivated soil have decreased during this dry, cold period. There was only 0.04 inch of rainfall in May, 1919, and 0.03 inch in June of the same year. The air temperatures were a mean maximum of 73.8°F., a mean minimum of 37.1°F. and a mean temperature of 55.4°F. during May; and for June a mean maximum of 72°F., a mean minimum of 34.6°F. and a mean temperature of 53.3°F.

The day temperature would not stop nitrate production but the lack of moisture was so great, and the actual moisture content of the virgin soil so much lower than that of the cultivated, that it is surprising that a small increase in nitrate in the virgin soil is recorded, where the cultivated soil has decreased in that respect.

A similar strange behaviour of the dry-land virgin soil will be noticed in figure 1, illustrating seasonal variation. This plot shows a rise in nitrates just at the time all the other four plots show a big fall, only this takes place at the height of the rainy season instead of in the dry winter period. This point will be discussed again.

TABLE 1

Preliminary studies

DATE	LOCALITY	PLOT NUMBER	SOIL MOISTURE	NITRIC NITROGEN	TREATMENT PER ACRE AND REMARKS
1919			*per cent*	*p.p.m.*	
June 1	Dry land, virgin soil near football field		7.0	0.48	Soil very hard and difficult to sample
			6.0	0.47	
June 30	Dry land, virgin soil near football field		3.0	2.9	
June 30	Soil from cultivated and irrigated land		2.7	0.4	This soil had not been irrigated for many months and had grown sorghums
June 10	Dry land, experiment plots, potato series	1	8.1	27.6	8 tons farm manure
		2	8.6	45.0	Control
		3	8.9	34.5	70 lbs. K_2SO_4, 150 lbs. dried blood, 200 lbs. super
		4	8.3	33.4	70 lbs. K_2SO_4, 200 lbs. super
		5	8.2	24.4	150 lbs. dried blood, 200 lbs. super
		6	7.0	38.6	Control
		7	6.9	32.6	150 lbs. dried blood, 70 lbs. K_2SO_4
		8	5.7	30.9	70 lbs. K_2SO_4
		9	6.9	38.6	200 lbs. super
		10	6.2	32.1	Control
		11	6.6	48.5	150 lbs. dried blood
July 11	Dry land, experiment plots, potato series	2	6.5	20.4	Control
		3	6.8	21.4	70 lbs. K_2SO_4, 150 lbs. dried blood, 200 lbs. super
		6	7.0	18.2	Control
		9	7.1	22.8	200 lbs. super
		11	7.3	22.7	150 lbs. dried blood
July 4	Dry land, experiment plots, maize section	5	5.0	5.3	150 lbs. super
		7	5.3	5.9	Control
		10	5.3	6.3	500 lbs. Saldanha Bay phosphate, 75 lbs. dried blood
		12	4.7	6.0	75 lbs. dried blood
		15	4.5	6.6	Control

If table 1 is consulted, it will be seen that on June 10 the soil of the dry-land potato plots had a good nitrate-nitrogen content, but that the five plots that were repeated a month later showed a distinct decrease in nitrates. The nitrates on five maize plots on an adjoining series show a much lower nitrate content than the potato plots. Both crops had been harvested in the middle

of May, but it is possible that the increased aeration caused by the plowing up of the potatoes may account for the greater nitrate content of the soil, although the samples were not taken in the plowed tracts, but between them. Again, potatoes do not use as much nitrogen as maize, and relatively the maize crop was better than the potato crop. There does not appear to be any definite, inverse relationship between the amount of nitrate found on the plots and the yields of the crop. These figures are at the disposal of the writer, but as they have not been published and are not his own work, they are not included in this paper.

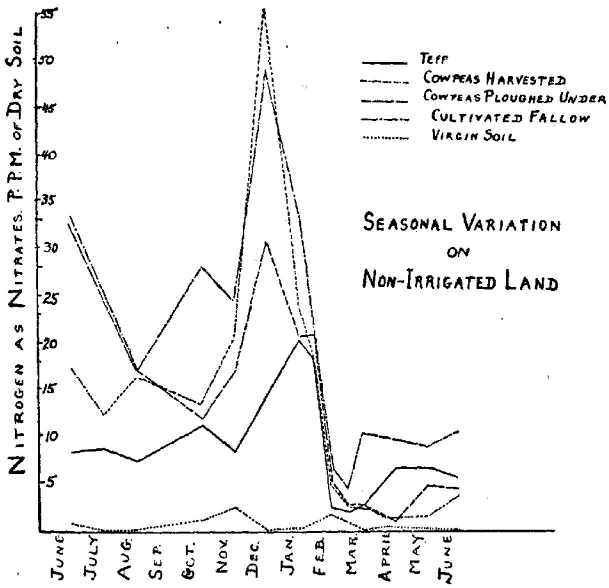

FIG. 1. SEASONAL VARIATIONS IN NITRATES, ON VIRGIN SOIL, CULTIVATED FALLOW LAND AND CULTIVATED CROPPED LAND, 1919–20 (table 3)

The nature of the fertilizers which the plots received does not appear to have affected the subsequent nitrate content; even the addition of 150 pounds of dried blood per acre has not increased the nitrate content above that of the check plots, with the exception of that on plot 11.

Plot 1, receiving 8 tons of stable manure per acre, does not show as good a nitrate yield as the controls. This was the first year that this land had been planted to a crop, and the physical condition of this plot, judging from the difficulty in sampling it, was not as good as that of the other plots, so this may be explained by lack of proper aeration. Lyon and Bizzell (28) record

higher nitrate contents in soil that had carried maize than unplanted soil, but the writer found 34.8 parts per million of nitric nitrogen on the fallow strip beside the maize plots at the end of the season, the highest of which contained 8.7 parts per million. Their figures also show that the potato soil was next highest in nitrates, while the writer's show that the potato soil was far ahead of the maize, and just about equal to the fallow soil beside it. A great deal of work would have to be done on this subject, though, to obtain results comparable to theirs in amount of data. An important fact must not be overlooked, i.e., on July 4, the maize plots contained less moisture than the potato plots did on July 11, and no rain had fallen in the meantime.

McBeth and Smith in Utah found that no increases in nitric nitrogen took place when the soil moisture content had reached 5 per cent (33).

They found too that the nitrifying power of soil that had grown potatoes was slightly better than that of soil which had grown maize, and that both were better than the fallow land in this respect.

II. SEASONAL VARIATION

On June 16, 1919, the first samples were taken on the dry-land fertilizer and crop rotation plots, with a view to studying the seasonal variation on non-irrigated land under Potchefstroom conditions. On account of other duties it was possible to take samples on the plots only once monthly. During the most active growing period for maize in February and March, however, sampling was done every two weeks. The soil on which these plots are situated is a reddish brown, fine sandy loam, which becomes very hard and compact in dry weather. It has a nitrogen content of 0.09 per cent and contains 6.7 per cent of organic matter.

By referring to tables 2 and 3 and figure 1 it will be seen that the study was carried on over the period of a year, on five different but adjacent pieces of land, which are designated as "teff," "cowpeas harvested," "cowpeas plowed under," "cultivated fallow" and "virgin soil." From the same tables and figure 6 it will be seen that seasonal variation was also studied for a shorter time on additional plots termed "maize after maize," "teff after maize," "uncultivated fallow," "cowpeas plowed under after teff."

There are four strips of land termed A, B, C, and D each divided into 17 $\frac{1}{20}$-acre plots, on which a rotation of maize, maize, cowpeas and teff is practised. On thirteen of the cowpea plots the crop is cut for hay, and on four of them it is plowed under as a green manure. As a system of fertilizing is also practised the samples for nitrate work were taken only on the control plots.

When this study was started in the winter of 1919 the range A called "teff" had just grown that grass for hay, on range B called "cowpeas harvested," the crop had been cut for hay, while on that termed "cowpeas plowed under," the cowpeas were used as a green manure. "Cultivated fallow" was a plowed and harrowed strip between A and B, or "teff" and "cowpeas," while the

virgin strip was along the outside of the plots. These names are followed throughout in order to avoid confusion, as the nitrates were determined on the same plots the whole year, but it must be borne in mind that the crop changed. For example, the "teff" section carried cowpeas, while the cowpeas were followed by maize in the middle of the season. Table 2 makes this clear.

By referring to figure 1 it will be seen that from June to July four of the five strips of land under observation show a decrease in their nitrate-nitrogen content, the "teff" only remaining about constant. This general decline corresponds somewhat with the downward temperature curve, for the cold dry months of June and July, as shown in figure 4.

Four of the plots show a continued decrease throughout August, "cowpeas harvested" alone showing an increase, which corresponds to the upward curve of the temperature record in that month. All during September and October,

TABLE 2

Planting and cultivating record

RANGE	SEASON 1918–19	SEASON 1919–20	PLOWED	PLANTED	RATE PER ACRE	CULTIVATED	HARVESTED
					lbs.		
A	Teff	Cowpeas	Nov. 14, 1919	Nov. 22, 1919	20	Dec. 12, 1919 Feb. 19, 1920	Feb. 4, 1920
B	Cowpeas	Maize	Nov. 14, 1919	Nov. 22, 1919	15	Dec. 12, 1919 Feb. 19, 1920	May 26, 1920
C	Maize	Maize	Nov. 14, 1919	Nov. 22, 1919	15	Dec. 12, 1919 Feb. 19, 1920	May 26, 1920
D	Maize	Teff	Nov. 14, 1919	Nov. 22, 1919	6	Nil	Feb. 20, 1920

however, this plot falls again almost to its July level. "Cowpeas plowed under" also decreased from June to the end of October, while during September and October the temperature rises, reaching its third highest crest of the year. There is also an increase during this time of the nitric nitrogen in the "virgiu," "teff" and "cultivated fallow" plots. There was 0.15 inch of rain in the second week of September, which does not even show in the soil-moisture curves (fig. 2), and can hardly be responsible for the gain of these three plots. The "teff" and "fallow" plots show a depression in November, while the virgin continues to rise. Both cowpea plots also show a steep rise in November, the first month of good rain. The virgin soil reached its highest level at the end of November. During December there is a steep rise for all except the virgin strip which is again declining. "Cultivated fallow," "cowpeas harvested" and "cowpeas plowed under" reach their highest level by the end of December, before the maximum temperature curve is reached in January. The "teff" plot is the only one which reaches its maxi-

TABLE 3

Seasonal variation in nitric nitrogen on non-irrigated land, dry soil; June, 1919, to June, 1920

PLOT AND RANGE	JUNE 16	JULY 25	AUGUST 27	OCTOBER 2	NOVEMBER 6	DECEMBER 9	JANUARY 9	FEBRUARY 4	FEBRUARY 26	MARCH 4	MARCH 17	APRIL 8	MAY 6	JUNE 8
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m*	*p.p.m.*
Virgin	1.0	Nil	Faint trace	1.0	2.3	Nil	Trace	Faint trace	1.6	Nil	Nil	0.4	Trace	Trace
Teff (A)	8.3	8.7	7.3	11.1	8.3	14.6	20.0	14.1	2.4	2.0	2.7	6.4	6.5	5.3
Cowpeas harvested (B)	17.4	13.3	16.2	12.5	20.1	55.3	24.0	18.4	5.0	2.5	2.1	1.2	1.4	3.7
Cowpeas plowed under (B)	32.6	16.1	17.1	11.9	16.9	30.9	20.6	20.7	5.2	2.8	2.6	1.0	4.7	4.5
Cultivated fallow	34.8	24.6	16.2	28.2	25.6	49.0	33.5	20.7	6.5	4.4	10.2	9.5	9.0	10.3
Uncultivated fallow							16.1					5.5	6.0	10.7
Maize after maize (C)							16.1	10.0	1.2	3.4	1.8	1.7	2.7	3.4
Teff after maize (D)							18.5	5.1	Trace	1.8	Nil	0.2	1.5	1.7
Cowpeas plowed under after teff (A)									4.9	5.3	4.9	16.3	13.2	11.1

TABLE 4

Moisture of non-irrigated soils, showing seasonal variation, June, 1919, to June, 1920

PLOT AND RANGE	JUNE 16	JULY 25	AUGUST 27	OCTOBER 2	NOVEMBER 6	DECEMBER 9	JANUARY 9	FEBRUARY 4	FEBRUARY 26	MARCH 4	MARCH 17	APRIL 8	MAY 6	JUNE 8
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Virgin soil	7.7	7.0	6.4	5.1	9.7	8.3	9.0	10.4	13.1	12.4	10.6	8.9	7.4	7.9
Teff (A)	6.4	5.9	5.2	3.5	9.5	10.8	10.1	10.6	13,1	11.4	11.0	9.9	8.9	8.9
Cowpeas harvested (B)	6.7	6.9	5.6	5.6	9.5	10.8	11.2	11.5	13.0	11.4	10.6	9.9	8.6	8.6
Cowpeas plowed under (B)	7.2	7.4	6.0	4.5	9.9	10.3	9.6	10.6	13.2	11.3	10.9	9.2	8.0	9.0
Cultivated fallow	8.8	9.5	7.7	7.0	10.6	8.8	8.9	11.5	13.9	12.5	11.6	11.3	10.7	10.7
Uncultivated fallow												5.9	10.1	10.4
Maize after maize (C)							9.1	11.3	12.9	11.6	9.7	7.9	8.1	8.2
Teff after maize (D)							8.2	9.4	12.0	10.8	9.1	7.4	6.0	7.9
Cowpeas plowed under after teff (A)									13.2	12.4	13.5	11.4	8.7	10.0

mum at the end of January, after the temperature maximum, while the three just previously mentioned are showing a steep downward fall. During the last few weeks of February there is a uniform and steep drop for all the plots, excepting the virgin. This sudden decrease in nitric nitrogen is shown also in the additional plots studied, and plotted in figure 6. The virgin soil attains a second crest at the end of February, while all the other plots are about at their lowest ebb.

This was a very active growing time, and it might at first be supposed that the nitrates had been strongly drawn upon by the crops, but by reference to table 2 it will be seen in range *A* called "teff" in figure 1, the cowpeas had

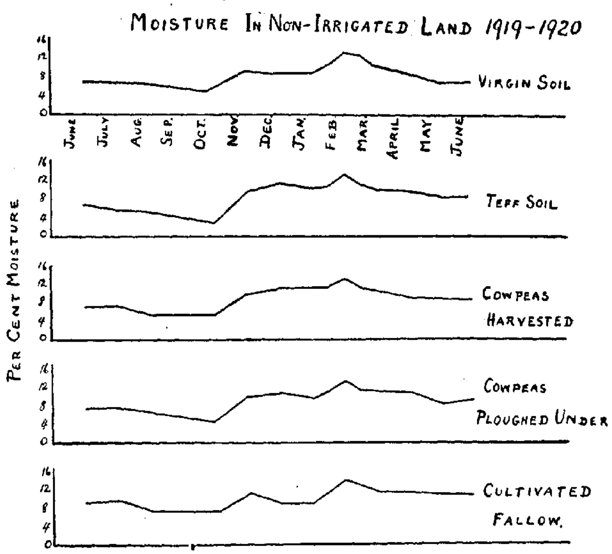

FIG. 2. MOISTURE CURVES OF THE STRIPS PLOTTED FOR NITRATES IN FIGURE 1 (table 4)

been harvested on February 4, 1920, and further the fallow land also shows this great decrease. On this account we cannot attribute the steep drop to crops. From the end of the first week in January to the end of the first week in February, there is a fairly steep drop in temperature, but not sufficient to retard nitrification greatly or account for the lessening of the nitrates. This sudden fall in nitrates, in January and February particularly, corresponds much more closely to the rainfall in those months, than it does to the drop in temperature. In fact, the nitrate curves on the whole fit the rainfall diagram better than they do the one for temperature. If the soil-moisture curves are consulted (fig. 2) it will be seen that there is a steep rise in November, after

which the greatest amount of nitrates was found. Again the highest crests in the soil-moisture curves correspond to the lowest in the nitric-nitrogen diagram. It was after the good rainfall in the first week in November that these plots show a good rise. There was again 1.8 inches of rain the last week in November, and this is followed by 0.7 inch the first week in December. There is then a 2-week period with only 0.09 inch of rain, the temperature is high, the soil moist, in fact conditions are ideal for nitrification. By the end of December the greatest increase is found in the nitrates, in spite of the 0.95

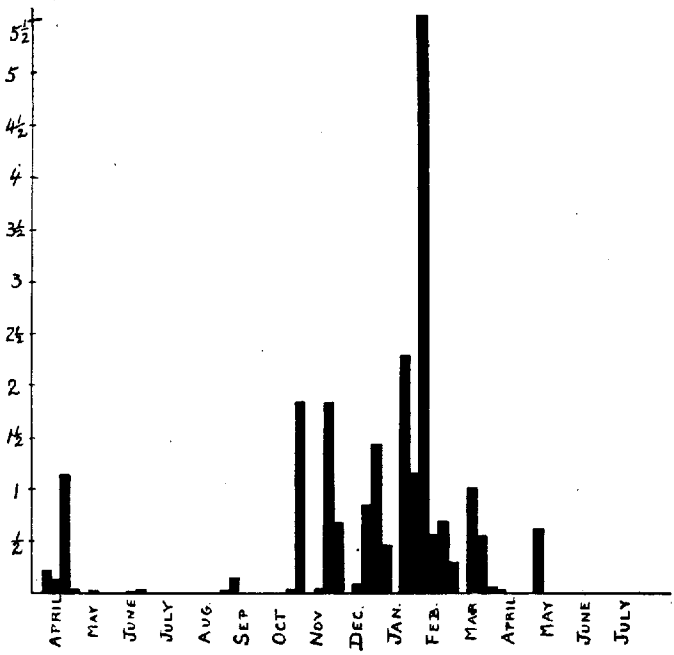

FIG. 3. WEEKLY RAINFALL IN INCHES, 1919–20

inch of rainfall in the last week, which may have washed some nitrates below the first food zone. The week after this highest crest in the nitrates was reached, i.e., the first week of January, there was a rainfall of 1.5 inches and it is after this and the 2.3 inches the last week in January that the rapid decrease in nitric nitrogen is noticed. The first week in February had 1.15 inches of rain and the second week was the rainiest of the season with 5.68 inches. It is after this downfall that there is the very uniform drop in six plots (fig. 1 and 6).

This decrease can be attributed largely to the heavy rains in February, and if table 15 is referred to, it will be seen that in cases 1, 2 and 4, representing depth samples taken at different parts of the plots under study, no nitrates are recorded in the fourth foot and in case 7, only traces of nitrates were found in the fifth foot in January.

No. 8, "maize after maize," was sampled on February 3, 1920, and contained 21 parts per million of nitric nitrogen in the first foot, 1.2 in the fourth, and nil in the fifth. On February 20 this same plot was again sampled, 6 inches of rain having fallen in the meantime, and only 5.3 parts per million were found in the first foot, while the fourth foot contained 12.7 parts per million and the fifth foot 8.4. The evidence is such as to leave little doubt

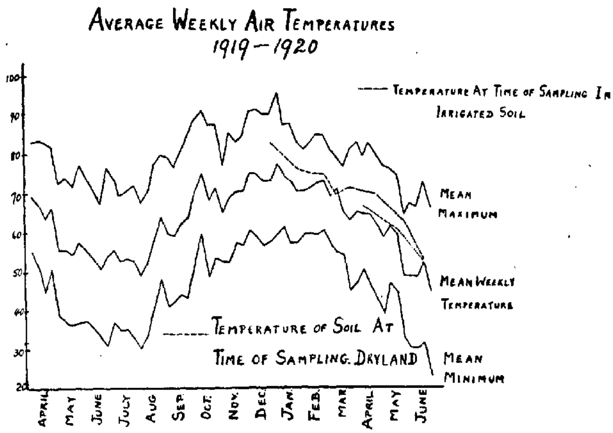

Fig. 4. Average Weekly Air Temperature, 1919–20. Also Soil-Temperature Curve for 6 Months on Dry-Land Soil at the Time of Sampling, and for 3 Months on Irrigated Land

that the very uniform and rapid decrease in the nitrates in February can be attributed to the heavy rains.

After this exceedingly rapid decrease there is still a further decrease until the middle of March, and during the latter two weeks of March the "cultivated fallow" has again risen 6 parts per million, and the "teff" strip, on which the cowpeas had been cut on February 4 also shows a rise. The virgin land with grass, and the cowpea strips growing maize decrease steadily, the latter two reaching about 1.0 part per million at the end of the growing season. There is no doubt that these decreases, while the bare strips are increasing, are due to the nitrates being used by the crops. After the end of April when the maize is ripe, there is again a rise on their nitrate content. The "maize after maize" plot (fig. 6) also shows the rise.

During the last week in May there were killing frosts, yet the two plots which had previously reached the highest two crests recorded in this investigation, show a decided rise after this and during the subsequently colder month of June. Figure 6 shows this rise, also on three of the four plots after some of the coldest weather experienced during the whole year, the uncultivated fallow making the most rapid rise. Five of the seven plots of the limed

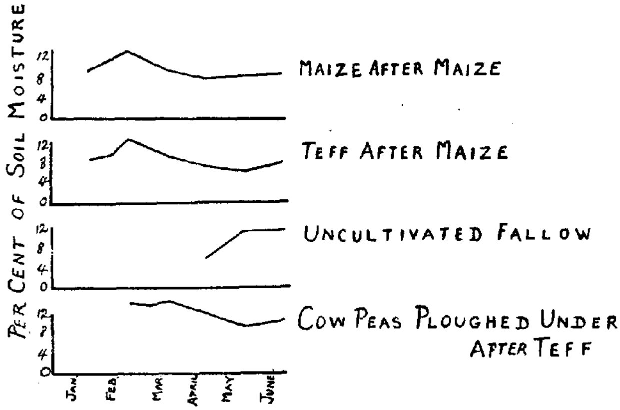

FIG. 5. MOISTURE CONTENT OF SOIL PLOTS STUDIED FOR NITRATES FOR A LESSER TIME

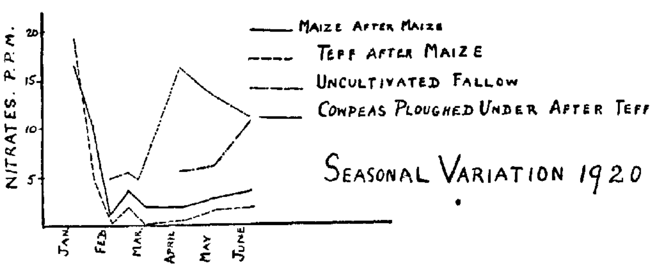

FIG. 6. THE NITRATE CURVES FOR THE SOIL PLOTS STUDIED FOR NITRATES FOR A LESSER TIME

potato series also show a very distinct rise in June. This may perhaps be explained by the biological theory of partial sterilization of Russell and Hutchinson which they apply to heated soils.

The frost, like the heat, may kill the protozoa and other enemies of nitrifying bacteria, which then become more active, and an increase in nitrates is found as a result. The low moisture content of the soil and the low temperatures prevailing at that time of the year, however, are not conducive to

very active nitrification. This is a point which the writer intends to investigate further as this rise in the nitrate curves corresponding to the lowest temperature curve, and the absence of rainfall is indeed striking.

The sudden rise in nitrates in November and December after the first good rains, also may be due to partial sterilization by drought and heat, e.g. the temperature of cultivated dry soil on October 19, 1920, was 35.5°C. and last year the soil was even drier.

Conn (7) obtained the highest counts of bacteria of the whole year from the frozen soil, and Brown and Smith (4) in the main confirm his work.

The behavior of the virgin soil

The virgin soil remains uniformly the lowest in nitric-nitrogen content throughout the year. It was also hard and compact even in the rainy season, and the most difficult to sample all the year round, the pick being used more often than the spade. Bad aeration may be put down as the factor most detrimental to nitrification. A strip of virgin land adjacent to that on which the virgin-soil samples were taken the whole year through, was sampled on January 13, 1920, just before plowing, and contained 1 part per million of nitric nitrogen. It was afterwards cultivated and planted to potatoes. Eighteen days later the check plot of this strip had increased to 39.8 parts per million, showing how effective is aeration combined with sufficient soil moisture and a high temperature for nitrification. The unplowed land again analyzed for nitrates at this time showed only a trace.

The virgin soil shows a steady and gradual rise in nitrate content from August, despite the low moisture content, which decreased until the end of October. In this respect the upward nitrate curve conforms with that of temperature rise. After the first November rains the curve rises at once more steeply, and attains its maximum at the end of that month, after a rainfall of 3.8 inches.

By the end of December there is only a trace of nitrate and this state of affairs continues all through January. In February there is again a rapid rise, and the virgin soil attains its second highest crest at the end of that month. This corresponds to the time that all the others dropped to almost their lowest nitrate content. The heavy rains were not absorbed by the compact soil as was done by the cultivated strips, and there was only a trace of nitrate in the soil which could be leached out, but the increased soil moisture stimulated nitrification. This may be explained also by the fact that oxygen in solution was carried in the soil by the heavy rains, giving the nitrifying bacteria more than they ordinarily obtained. By the end of March the nitrates of the virgin soil have again reached the zero level and do not rise from that for the rest of the year. The last downward curve in March corresponds to that of the other plots growing maize. There is no doubt that the decrease of the small amount of nitric nitrogen on the virgin grass-land in December, and the very low amount during January, can be attributed to

the veld grasses, which grew rapidly after the spring rains, and had nearly all matured by the end of January. After that time the nitrates rose again, but the writer makes no attempt to explain the subsequent fall in March. Lyon and Bizzell have shown that timothy, and also mixed grasses, like *phleum pratense*, *agrostis alba*, and *poa pratensis*, have a depressing effect on nitrification in the soil both at the time of growth and subsequently (27, 29, 31). The writer's records for the year show that the virgin soils with mixed native grasses and the soil growing teff also were lowest in nitrates.

June nitrate content, 1919 and 1920

The final nitrate content of none of the five plots was so high in June, 1920, as it was in June, 1919. This may be accounted for by the fact that April of 1920 was very dry, whereas there was a good rainfall of 1.14 inches in April, 1919, when the growing season was over, and the temperature was still fairly high, hence there was increased nitrification. Neither was there subsequent rain to leach out the nitrates. It is true there was 0.6 inch of rain in May, 1920, and only a trace in May, 1919, but a week after the rain had fallen the temperature dropped rapidly and there were killing frosts, while after the rain in April, 1919, there were several weeks of much warmer weather. Attention has already been drawn to the upward tendency of the nitrates after these frosts, but the cold and dry soil was sufficient to prevent the nitrates amounting to what they did in 1919.

It is worthy of note that the moisture curve from the uncultivated fallow, which had developed a hard superficial crust, ended the season as high as that of the cultivated fallow, and higher than any other plots.

Soil temperature

In figure 4 will be found plotted the soil-temperature curves, the average temperature for all the plots for the day being taken. It will be noticed that the soil temperature with one exception lies between the mean air temperature and the mean maximum air temperature. Lyon and Bizzell's (28) maximum soil-temperature curves practically correspond to the mean-air temperature curves. The writer's figures do not represent maximum temperatures, but are the temperatures taken at 6 inches at the time the sample was obtained and will be close to the maximum temperatures as they were procured between 10 a.m. and 3 p.m. The difference in temperature recorded on the same day is due chiefly to difference in time between the samples. The samples were always all taken on the same day, but not always in the same order.

Comparisons on seasonal variation

If the curves in figure 1 are compared with those of Russell and Appleyard (38), it will be noticed that they show both steeper rises and falls, especially in December and January, corresponding to June and July in the northern

hemisphere. There is also a rise in March and April corresponding to the rise at Rothamsted in September and October, but whereas all the curves show a drop in December, the majority of the writers show a distinct rise in June, the corresponding winter months here. December in the north is cold and wet, whereas June here is cold and dry. The rainfall curves throughout the year show a much more even distribution at Rothamsted than at Potchefstroom, but our rains come during the warm and growing season and so there ought to be maximum nitrification at that time. Our plots corresponding to their unmanured ones rise much higher, some of them four or five times as high, but also fall lower in nitrate content. The nitrates here are produced more quickly; their highest figure in unmanured land is 17 parts per million, ours is 49 parts per million on cultivated fallow, and 55 parts per million on land that had carried cowpeas. Nitrification under these conditions seems

TABLE 5

Soil temperatures at the time of sampling, 1919–20

PLOT AND RANGE	DECEMBER 9	JANUARY 9	FEBRUARY 4	FEBRUARY 28	MARCH 4	MARCH 17	APRIL 8	MAY 6	JUNE 8
	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
Teff (A)	27.0		24	24.5	21.5			17.5	12.2
Cowpeas harvested (B)	26.5	25.0		24.2	21.0	22.0	20.0	18.0	10.8
Cowpeas plowed under (B)		26.0		24.0	21.3	22.0	20.4	17.0	11.1
Cultivated fallow	28.0	25.0		25.2	21.0	22.4	21.0	18.0	12.7
Virgin soil	28.0			23.4	21.8		20.0	16.5	10.0
Uncultivated fallow							23.6	17.5	
Maize after maize (C)			24	24.3	23.5			18.0	11.0
Teff after maize (D)			24	25.5	23.0		24.0	18.0	11.5
Cowpeas plowed under after teff (A)				26.0	21.3		21.3	16.5	11.5

more limited by moisture, the nitrate curve is not so even, but when moisture is adequate, nitrification is more efficient. On heavily dunged land Russell reports 37 parts per million of nitrate, but rarely above 23 parts per million. Tulaikoff on fallow land in a semiarid portion of Russia, as a 5-year average, did not find more than 22 parts per million in July (46). This approximates more closely the Rothamsted figures than the writer's.

Buckman in Montana shows an April and May crest corresponding to our October and November rises, and high crests in September and October corresponding to our March and April rise (5). He records nitrates reaching the maximum in October, whereas here the maximum was reached in December, corresponding to June there. He records amounts of 66 parts per million of nitric nitrogen. Lyon and Bizzell (28) show under humid conditions at Ithaca, N. Y., a very steep rise to 190 parts per million for bare unfertilized

soil and then a big drop again but not nearly as steep as that which the writer has here recorded. This maximum in July would correspond to ours in January, when our maximum was actually in December a month earlier.

Their work in general shows much higher quantities of nitric nitrogen in the field than the writer obtained even by incubating untreated South African soils under ideal conditions.

The amounts in general which the writer has recorded are higher than those of Stewart in Utah, and similar to Australian results, obtained by Scott and Robertson (36). Green, under Leipzig conditions (10), found a rise of nitrates from August to October, a fall to November, and then a marked rise in December, which he attributes to the mild winter of 1912–13. The latter corresponds to our June rise. The highest amount of nitric nitrogen which he records is 100 parts per million, but this is after incubation.

Compared with nitrification in Rothamsted soils the writer can endorse Watt's opinion that it is more active in Transvaal soils, but when compared with the much larger amounts found at the Cornell Experiment Station under humid conditions, the writer realizes that the above view cannot be applied to humid soils in general, as was done by Watt.

From the work here recorded it would seem that nitrification was much more active for a part of the year at least than at Rothamsted in the corresponding time, but that it was much inferior to nitrification at Ithaca, N. Y., as recorded by field samples.

III. A STUDY OF THE NITRIFYING POWERS OF TWO SOILS

This study was carried out with the object of ascertaining whether, as was supposed, the nitrifying power of a soil that had been cultivated and irrigated for 14 years, was better than that of the same soil type a few hundred yards away that had never been irrigated or cultivated in that period. The virgin soil type is on a slope above the irrigation furrow and about 50 yards from it. The irrigated soil has grown wheat, oats, barley, maize, kaffir corn, mangels, lucerne, cowpeas and beans.

Samples of both soils were carefully taken to the depth of 1 foot, more soil being sieved and mixed than was required to fill two series of 20 half kerosene[1] tins each holding 18 pounds of dry soil.

The different amounts of lime and blood meal were mixed as per tables 6 and 7 after the soil had become quite air-dry. The weighed mixtures were rolled together on sheets of clean paper and put into their respective tins.

On July 1, 1919, all the 40 tins were watered at the rate of 15 per cent of the dry weight of the soil. All the tins were subsequently watered with equal amounts, the intervals depending largely on the rate of evaporation. The

[1] Kerosene, or paraffin as it is called in South Africa, is shipped in 4½-gallon tins. Petrol, or gasoline, comes in the same manner. These tins are used for plants, buckets and roofing material by many of the natives. The uses these tins are put to are innumerable.

TABLE 6

Nitric nitrogen in a non-irrigated virgin soil

NUMBER	JULY 29, 29TH DAY	OCTOBER 20, 112TH DAY	JANUARY 5, 189TH DAY	MARCH 12, 256TH DAY	TREATMENT
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	
1	3.4 s	12.3 s	27.1 s	47.1 s	Control
2	7.7 g	21.7 g	36.1 g	51.0 g	Limestone, 10 gm.
3	5.1 s	24.5 s	31.9 s	56.4 s	Limestone, 10 gm.
4	10.9 g	46.0 g	65.5 g	75.9 g	Limestone, 30 gm.
5	9.7 s	51.6 s	82.8 s	104.3 s	Limestone, 30 gm.
6	5.2 g	22.6 g	33.8 g	45.1 g	Control
7	6.0 g	38.6 g	56.2 g	55.1 g	Dried blood, 2 gm.
8	3.8 s	52.2 s	44.8 s	58.5 s	Dried blood, 2 gm.
9	6.8 s	24.3 s	75.2 s	79.3 s	Dried blood, 4 gm.
10	5.8 g	40.0 g	76.7 g	76.4 g	Dried blood, 4 gm.
11	4.4 s	29.0 s	64.4 s	83.6 s	Dried blood, 2 gm.; limestone, 10 gm.
12	8.2 g	25.8 g	42.3 g	47.4 g	Dried blood, 2 gm.; limestone, 10 gm.
13	12.0 s	82.6 s	64.0 s	96.4 s	Dried blood, 2 gm.; limestone, 30 gm.
14	10.3 g	61.1 g	54.9 g	83.0 g	Dried blood, 2 gm.; limestone, 30 gm.
15	5.4 s	30.9 s	43.1 s	51.9 s	Control
16	4.3 g	54.7 g	46.3 g	55.4 g	Dried blood, 2 gm.; limestone, 10 gm.
17	6.6 s	66.9 s	54.9 s	52.4 s	Dried blood, 2 gm.; limestone, 10 gm.
18	7.7 g	33.4 g	51.3 g	55.0 g	Dried blood, 2 gm.; limestone, 30 gm.
19	6.0 s	74.9 s	63.5 s		Dried blood, 2 gm.; limestone, 30 gm.
20	4.0 g	17.2 g	33.1 g	50.3 g	Control

s—Signifies tin was on wooden stand.
g—Signifies tin was on dry ground below stand.

TABLE 7

Nitric nitrogen in an irrigated and cultivated soil

NUMBER	JULY 29, 29TH DAY	OCTOBER 20, 112TH DAY	JANUARY 5, 189TH DAY	MARCH 12, 265TH DAY	TREATMENT
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	
21	3.2 g	5.9 g	13.6 g	13.8 g	Control
22	3.3 s	10.3 s	18.0 s	19.2 s	Limestone, 10 gm.
23	3.6 g	7.3 g	12.8 g	14.2 g	Limestone, 10 gm.
24	3.8 s	13.5 s	21.3 s	24.7 s	Limestone, 30 gm.
25	4.5 g	12.7 g	21.5 g	38.0 g	Limestone, 30 gm.
26	3.5 s	11.7 s	13.7 s	20.4 s	Control
27	9.7 s	24.1 s	27.4 s	39.1 s	Dried blood, 2 gm.
28	7.4 g	19.2 g	31.4 g	38.2 g	Dried blood, 2 gm.
29	16.5 g	42.3 g	32.4 g	59.4 g	Dried blood, 4 gm.
30	19.2 s	131.4 s	46.6 s	64.8 s	Dried blood, 4 gm.
31	14.9 g	32.0 g	37.4 g	40.2 g	Dried blood, 2 gm.; limestone, 10 gm.
32	11.0 s	28.3 s	35.4 s	51.7 s	Dried blood, 2 gm.; limestone, 10 gm.
33	13.2 g	21.6 g	27.2 g	24.8 g	Dried blood, 2 gm.; limestone, 30 gm.
34	9.0 s	28.2 s	37.1 s	60.6 s	Dried blood, 2 gm.; limestone, 30 gm.
35	4.0 g	9.1 g	12.8 g	21.1 g	Control
36	11.3 s	25.4 s	38.8 s	86.1 s	Dried blood, 2 gm.; limestone, 10 gm.
37	11.2 g	42.4 g	29.4 g	37.9 g	Dried blood, 2 gm.; limestone, 10 gm.
38	10.6 s	33.1 s	40.7 s	46.8 s	Dried blood, 2 gm.; limestone, 30 gm.
39	13.4 g	24.6 g	26.0 g	97.8 g	Dried blood, 2 gm.; limestone, 30 gm.
40	2.2 s	9.8 s	18.2 s	26.7 s	Control

s—Signifies tin was on wooden stand.
g—Signifies tin was on dry ground below stand.

tins were exposed all the time to all temperatures and weather conditions, except that they were protected from rain by a galvanized iron roof. The wooden stands were not sufficient to accommodate all the tins, so about half of them had to be placed on the dry earth below. Samples of cultivated and virgin soil were placed alternately, two duplicates not being in the same locality, e.g., if one check were on the ground the other would be on the stand. An effort was made in this manner to remedy local advantages, such as those of temperature. That the differences between the soil temperature on the stand and in the tins on the ground was very marked, is well shown in table

TABLE 8

Soil temperatures in tins on stand and on the ground

TIME	TIME ON STAND	TINS ON GROUND
a.m.	°C.	°C.
7.00	9.7	12.0
7.30	12.0	12.6
8.00	16.0	14.7
8.30	18.5	16.5
9.00	24.5	19.3
9.30	29.9	20.0
10.00	33.4	21.0
10.30	33.7	22.3
11.00	35.9	23.5
11.30	35.8	24.1
12.00	35.5	25.0
p.m.		
12.30	35.0	25.8
1.00	34.5	26.5
1.30	34.0	27.6
2.00	33.7	27.6
2.30	33.5	28.0
3.00	33.0	28.3
3.30	32.8	28.1
4.00	32.1	27.9
4.30	31.5	27.5
5.00	31.0	27.2

7, which is a comparative record of the soil temperatures during one day taken at half-hourly periods from 7 a.m. to 5 p.m. In the case of two tins of similar treatment, the one on the stand usually had a higher nitrate content on account of the more favorable temperature.

The nature of the soils

Both soils were sandy loams in type but the virgin soil is lighter and more reddish in color than the cultivated soil, which is brown. The virgin soil lost on ignition 5.5 per cent, the cultivated 4.6 per cent. The virgin soil by the Veitch method showed a lime requirement of 1800 pounds per acre,

whereas the cultivated soil was slightly alkaline in reaction, although no free carbonate could be detected. This soil had probably originally a lime requirement the same as the virgin, but it has been irrigated for 14 years with a water of high lime content, and has been made slightly alkaline.

In tins no. 11, 12, 13, 14, 31, 32, 33 and 34, the limestone and blood meal were applied and mixed separately with the soil. In no. 16, 17, 18, 19, 36, 37, 38 and 39, the limestone and blood-meal were mixed together intimately for several hours before being applied and mixed with the soil.

It is a saving in labor to the farmer if he can mix together two substances before they are applied to the land, instead of applying them separately. Dry blood-meal and dry carbonate of lime should not react harmfully if mixed together for only a short time before application, and the writer was of the opinion that apart from saving labor, the practice might bring about more efficient nitrification. The above mixing was done to give some definite confirmation of this opinion if possible.

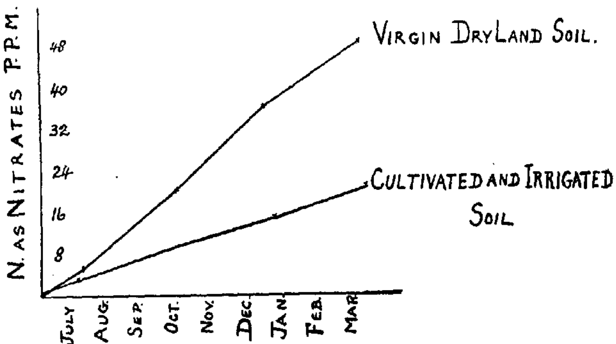

FIG. 7. NITRATE CURVE PLOTTED ON AVERAGE RESULTS OF THE UNTREATED SOILS

The influence of our winter temperature on nitrification

The temperature for the most part of the first period to July 29 was low at night, usually below 36°F., but did not drop to freezing. The maximum day shade temperatures, on the other hand, were in the vicinity of 72°F. At the beginning of the experiment there was no trace of nitrates in the virgin soil, while the cultivated contained only 0.5 part per million. At the end of 29 days there was a considerable increase in nitric nitrogen in both soils, and by referring to figure 7, it will be seen that the virgin and cultivated controls show a steady rise through July, August, September and October, the months during which there was very little activity in the field plots, as shown in figure 1. It would seem from these facts that it is moisture and not temperature that is the chief limiting factor for nitrification during the cold dry months in the Transvaal.

General discussion of results

In the first period the virgin soil is slightly ahead of the cultivated, as regards untreated soil, and also where lime has been applied. In that first period of 29 days the cultivated soil has shown superior powers of nitrification wherever blood-meal has been applied. The lime requirements of the two soils were so different that it would be expected that the virgin soil would give much better results from its application.

During the next three months the temperature has steadily increased and so have the nitrates, both in the controls and in the treated tins. The nitrates in the virgin soil are now almost without exception well ahead of those in the cultivated soil, and they maintain their superior lead through the 8½ months that the experiment was continued.

TABLE 9

Data of tables 6 and 7 in condensed form

NUMBER	JULY 29		OCTOBER 20		JANUARY 5		MARCH 12		TREATMENT
	Virgin	Cultivated	Virgin	Cultivated	Virgin	Cultivated	Virgin	Cultivated	
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	
1	4.5	3.2	20.7	9.1	34.2	14.5	48.6	20.5	Control
2	6.4	3.4	23.1	8.8	34.0	15.4	53.7	16.6	Limestone, 10 gm.
3	10.3	4.1	53.8	13.1	74.1	21.4	89.1	31.3	Limestone, 30 gm.
4	4.9	8.5	45.4	21.6	50.5	29.4	56.8	38.6	Dried blood, 2 gm.
5	6.3	17.3	32.1	86.8	75.9	39.5	77.8	62.1	Dried blood, 4 gm.
6	6.3	12.9	27.3	30.1	53.3	36.4	65.5	46.0	Dried blood, 2 gm.; limestone, 10 gm.
7	11.2	11.1	71.8	24.9	59.4	32.1	89.2	42.7	Dried blood, 2 gm.; limestone, 30 gm.
8	5.4	11.2	60.8	33.9	50.6	34.1	53.9	62.0	Dried blood, 2 gm.; limestone, 10 gm.
9	6.8	12.0	54.1	28.8	57.4	33.3	*55.0	72.3	Dried blood, 2 gm.; limestone, 30 gm.

* Determination from only one tin, accident to the other.

The maximum amount of nitric nitrogen obtained in the virgin controls corresponds closely to the maximum obtained in the fallow land of the same soil type in the field. In the tins this amount of nitric nitrogen, 48 parts per million, was reached by a steady upward curve in a period of 8½ months terminating in March, 1920.

The cultivated fallow in the field increased 24 parts per million in 4 weeks after the rain, and attained its maximum of 49 parts per million at the end of December, 1919. In the middle of March, 1920, it was down to 4.5 parts per million. The maximum under field conditions seems to be reached much more quickly. This may be due to partial sterilization by the hot sun on a dry soil. Of course, during the greater part of the day the tins were in the shade, and such high soil temperatures as those obtaining in the field would not prevail generally.

In order to appreciate better the differences shown in tables 6 and 7, these data have been condensed and recorded in abbreviated form in table 9. The figures represent the averages of two tins each, and of four controls. The data are represented graphically in figure 8.

In no. 6 and 7, dried blood and limestone were applied separately. In no. 8 and 9 dried blood and limestone were mixed together before application.

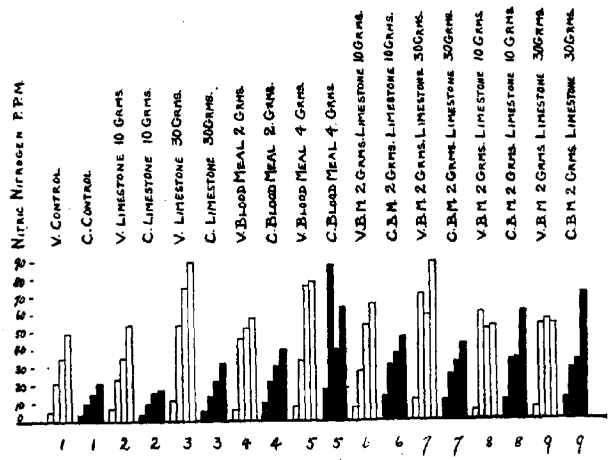

FIG. 8. GRAPHIC SUMMARY OF THE EXPERIMENT ON THE INFLUENCE OF TEMPERATURE ON NITRIFICATION

Unshaded graphs represent the virgin soil; shaded, the cultivated soil

A discussion of table 9 and figure 8

Both figures 7 and 8 show that in general nitrification was better in the soil from the non-irrigated virgin land than in that from the irrigated and cultivated land. This depression of nitrification by cultivation and irrigation was contrary to what the writer had expected to find.

Since his experiment has been completed, the writer finds that McBeth and Smith in Utah (13) also conclude that the application of irrigation water reduced the nitrifying powers of the soils as determined by laboratory methods.

On the virgin soil better results were obtained from blood-meal and limestone when applied separately than when mixed together before application. On the irrigated and cultivated soil on two occasions better results were obtained by mixing the blood-meal and limestone; on two others this was not the case, but the final determinations of nitrates in no. 8 and 9 are well ahead of those in no. 6 and 7.

It would seem from the data here obtained that the practice of mixing blood meal and limestone together before applying to the land cannot be recommended because of its uniform beneficial effects on nitrification, although it may be as a means of saving labor.

Limestone alone at the rate of 10 gm. per tin, or 3057 pounds per acre, does not appear to have benefited nitrification in either the virgin or the cultivated soil, but when three times that amount is applied, nitrification is greatly increased over the controls, about three-fourths more on the virgin and nearly as much on the cultivated soil.

The cultivated soil is alkaline to start with and the virgin has a lime requirement of 1800 pounds per acre. It is strange that they benefit about equally from heavy liming.

Two grams of blood meal with the virgin soil did not produce as much nitrate as 30 gm. of limestone. In the cultivated soil, on the other hand, 2 gm. of blood meal produced more nitrate than 30 gm. of limestone. Even 4 gm. of blood meal (1200 pounds per acre) in the virgin soil did not produce as much nitrate as 30 gm. of limestone, whereas in the cultivated soil more than twice as much was produced. These amounts are far above the limits that would be used in practice in South Africa, but still the results from them are illuminating.

The addition of 10 gm. of limestone to the soil containing 2 gm. of blood meal gave slightly increased amounts of nitric nitrogen with both soils, whereas the addition of 30 gm. in tins containing 2 gm. of blood meal greatly increased nitrification in the virgin soil, but appears to have depressed it somewhat in the cultivated soil.

From the data obtained in this experiment the following conclusions seem possible:

1. The outdoor winter temperatures of July and August, 1919, were not sufficient to stop nitrification, while the soil moisture was adequate.

2. It seems as if nitrification is kept in check in our cold dry winters more by lack of moisture than by actual cold.

3. Nitrates accumulated in the winter and increased for 8½ months continuously, because the soil was protected from leaching losses by rain.

4. In some cases there seems to be a benefit shown by increased nitrification in addition to a saving of labor when blood meal and limestone are mixed together before being applied to the soil.

5. Cultivation and irrigation for 14 years seemingly depressed the nitrifying power of the soil studied.

6. Limestone applied in amounts largely in excess of those used in practice greatly stimulates nitrification, even in a slightly alkaline soil. This does not imply, however, that superior crop yields could be expected from such an application, for in common with most South African soils, the crop yields would probably be limited by lack of phosphates. Such applications, although largely stimulating nitrification, under our conditions would mean a monetary loss.

IV. THE NITRIFICATION OF COWPEAS USED AS A GREEN MANURE

Toward the end of January, 1920, on the cultivated and irrigated land from which soil had been taken for the last study, but on another portion of the field, 24 $\frac{1}{100}$-acre plots were prepared. The soil in this section is a brown sandy loam, with 5.9 per cent of organic matter, and 0.1 per cent of nitrogen. Whereas on the last study dried blood was used to ascertain the soil's nitrifying power, in this case cowpeas were taken. It is the policy of this station

TABLE 10

Nitrification of cowpeas with various amounts of lime

Number	February 11				March 1		April 14		May 20		June 22	
	NH_3	Nitrites	Nitrates	Soil moisture	Nitrates	Soil moisture	Nitrates	Soil moisture	Nitrates	Soil moisture	Nitrates	Soil moisture
	p.p.m.	*p.p.m.*	*p.p.m.*	*per cent*	*p.p.m.*	*per cent*	*p.p.m.*	*per cent*	*p.p.m.*	*per cent*	*p.p.m.*	*per cent*
1	0.3	Nil	2.6	14.5	1.4	14.5	6.3	11.4	5.2	11.3	5.9	11.2
2	0.7	Nil	5.4	15.1	6.8	14.8	6.3	11.2	8.8	11.3	8.1	10.7
3	1.0	Nil	8.9	14.5	12.5	14.0	11.6	10.9	18.2	12.1	10.3	10.1
4	1.8	Nil	6.3	14.2	9.4	14.3	8.1	11.5	14.3	10.9	11.1	10.2
5	0.8	Nil	8.6	13.4	7.1	13.7	5.9	10.4	13.6	10.5	14.3	10.5
6	0.9	Nil	7.0	13.8	9.0	13.4	8.6	11.6	20.7	10.3	11.4	10.3
7	0.9	Nil	9.1	13.8	10.0	13.3	5.7	10.5	14.1	11.0	12.3	9.5
8	0.7	Nil	8.5	14.1	5.5	14.3	9.1	10.7	12.1	10.7	12.4	9.6
9	0.5	Nil	4.7	12.9	1.4	14.4	7.8	12.0	11.6	11.6	8.5	10.1
10	1.3	Nil	7.0	13.5	5.5	14.6	7.5	12.3	10.9	10.7	6.2	9.4
11	1.4	Nil	2.6	13.1	4.3	14.7	4.2	11.1	9.3	10.9	8.3	9.9
12	0.8	Nil	4.0	15.4	5.3	14.8	9.2	11.6	13.7	11.4	10.8	9.8
13	2.7	Nil	6.2	13.2	7.2	16.1	11.5	11.0	10.1	10.9	10.3	10.0
14	2.5	Nil	2.5	13.5	5.7	14.9	6.4	11.8	10.3	11.7	10.0	9.2
15	1.3	Nil	4.4	12.1	5.3	14.2	8.6	11.6	9.5	11.4	14.1	8.1
16	2.4	Nil	3.5	12.0	4.4	14.2	6.4	11.7	13.1	11.4	9.6	10.5
17	1.1	0.1	5.7	13.4	4.7	15.4	9.0	12.3	11.5	11.9	11.4	10.7
18	2.1	Nil	3.6	11.8	4.1	15.6	5.8	12.8	6.6	12.2	9.4	10.6
19	2.3	Trace	2.9	14.6	5.2	15.7	8.6	12.4	12.7	12.2	12.4	10.7
20	2.2	Trace	4.9	14.0	7.6	13.7	7.2	11.6	17.8	11.0	15.0	10.2
21	Trace	Nil	4.9	14.4	5.8	15.0	8.7	12.6	10.2	10.8	10.5	10.7
22	Trace	Trace	3.5	14.3	5.7	14.8	7.1	12.4	14.9	11.7	16.1	9.5
23	Trace	Nil	3.8	13.5	5.1	14.6	7.0	12.3	11.5	11.4	14.6	8.4
24	Trace	Trace	4.3	13.9	6.3	14.7	10.0	12.0	11.5	11.3	11.3	9.9

to advocate the use of green manuring in preference to all other nitrogenous fertilizers, and it was felt that some observations in this area on the decomposition of our peas, and the amount of nitrate formed under field conditions might be of value.

An endeavor also was made to ascertain whether ground limestone or slaked lime was the better in bringing about decomposition and nitrate formation, and what quantities of these limes it was best to use.

Just as in the last study an attempt was made to find out if mixing lime and blood meal together gave increased nitrification in addition to saving

labor, so here the mixing of cowpeas and lime was tried. Lime was applied to the soil and turned under, then the green-cut cowpeas, just beginning to form pods, were applied and turned under. This treatment was compared

TABLE 11

Treatment and temperature of $\frac{1}{1000}$-acre plots

NUMBER	TREATMENT PER ACRE	APRIL 14		MAY 20		JUNE 22	
		°C.	Time	°C.	Time	°C.	Time
1	Control	17.0	9:35	14.0	9:20	10.0	9:15
2	2 tons cowpeas	18.0	9:55	14.0	9:30	10.5	9:30
3	2 tons cowpeas; 2000 lbs. $CaCO_3$ applied before cowpeas	19.0	10:20	14.0	9:42	10.0	9:40
4	2 tons cowpeas; 2000 lbs. $CaCO_3$ mixed with cowpeas	19.0	10:40	14.0	10:05	10.0	9:50
5	2 tons cowpeas; 1000 lbs. $CaCO_3$ applied before cowpeas	19.0	11:00	14.5	10:45	11.0	10:05
6	2 tons cowpeas; 1000 lbs. $CaCO_3$ mixed with cowpeas	19.0	11:15	14.5	10:55	10.5	10:15
7	2 tons cowpeas; 500 lbs. $CaCO_3$ applied before cowpeas	19.0	11:30	14.7	11:05	11.0	10:25
8	2 tons cowpeas; 500 lbs. $CaCO_3$ mixed with cowpeas	19.3	11:45	15.0	11:15	11.0	10:35
9	2 tons cowpeas; 2000 lbs. $Ca(OH)_2$ applied before cowpeas	19.3	12:50	15.5	11:35	11.0	10:45
10	2 tons cowpeas; 2000 lbs. $Ca(OH)_2$ mixed with cwpeas.	20.8	2:05	15.5	11:45		
11	Control	20.8	2:17	16.0	12:00		
12	2 tons cowpeas; 500 lbs. $Ca(OH)_2$ applied before cowpeas	20.0	2:30	16.0	12:05		
13	2 tons cowpeas; 500 lbs. $Ca(OH)_2$ applied before cowpeas	20.0	3:00	17.0	2:15		
14	2000 lbs. $CaCO_3$	21.0	3:08	17.0	2:18		
15	1000 lbs. $CaCO_3$	20.8	3:30	17.5	2:30		
16	500 lbs. $CaCO_3$	21.0	3:40	17.5	2:45		
17	2000 lbs. $Ca(OH)_2$	21.0	3:50	17.5	3:00	12.6	2.10
18	500 lbs. $Ca(OH)_2$	20.5	2:05	17.5	3:15	13.0	2:20
19	2000 lbs. $Ca.CO_3$	18.0	*9:25	17.5	3:25	13.5	2:30
20	1000 lbs. $Ca.CO_3$	18.5	9:40	18.0	3:40	14.0	2:40
21	500 lbs. $Ca.CO_3$	19.0	10:00	17.5	3:45	12.5	2:50
22	2000 lbs. $Ca(OH)_2$	19.8	10:15	17.5	4:00	13.5	3:00
23	500 lbs. $Ca(OH)_2$	20.1	10:25	17.0	4:10	13.6	3:15
24	2 tons cowpeas	20.4	10:41	17.0	4:15	13.0	3:25

* Samples and temperatures from here onward taken April 15.

with the process of applying cowpeas to the soil, sprinkling them with lime, and turning under the two together.

The detailed treatment of each plot is given in table 11, and table 10 contains the amounts of nitrate present on five different dates and the moisture content of the soil at those times.

On January 28, 1920, the green cowpeas were turned under on the $\frac{1}{100}$-acre plots, and two weeks later on February 11 samples were taken for the first determinations. Two 1-foot-deep holes were made in each plot and the samples from the two mixed together. From the mixture a representative sample was taken. Determinations of ammonia, nitrites and nitrates on February 11 were made on every sample taken.

As only traces of nitrites were found and the quantity of ammonia was so small at the first sampling, no more of these determinations were made. It rained so heavily shortly after the cowpeas were turned under, and at so many other times during the two weeks previous to the first sampling, that the cowpeas were completely decomposed, only occasional traces of the fibre of the stem being found. It is unfortunate that a time should have been chosen, which has subsequently been shown in figure 1 to be the worst in the year for finding nitrates in the first foot of soil. There is no doubt from the depth samples taken in spots not far removed from these $\frac{1}{100}$-acre plots, that the nitrates were carried down below the feeding zone of most roots. It was quite impracticable to take depth samples, as with pick and spade, the implements at the writer's disposal, nothing would have been left of a $\frac{1}{100}$-acre plot after having been once sampled below the third foot.

It is possible that the data would have been more valuable, if the season had not been quite so wet. The same soil type under the protected conditions prevailing in the last study gave much higher results in nitric nitrogen.

In order to study the 5 months' data to better advantage, they have been condensed in table 12.

These data are given in graphic form in figure 9 which shows the amount of nitric nitrogen in parts per million in each plot for the five dates of sampling. The inset shows the soil temperature steadily decreasing throughout three months. If reference is made to figure 4, it will be seen that these soil temperatures for the dates plotted fall in between the mean air temperatures and soil temperatures of the dry-land plots. The difference in soil moisture between the two series of plots is sufficient to account for this difference in temperature. On Juno 22 the soil temperatures of the two series coincide and their soil-moisture contents also are very similar.

Two tons of green cowpea hay per acre did not increase the nitrate content much above that of the controls. The application of 1 ton of limestone before the cowpeas greatly increased nitrification over the first two mentioned plots and also gave better results than turning under the cowpeas and limestone together. With 1000 pounds of limestone, though, slightly better results were obtained by turning under the cowpeas and limestone together, than by applying them separately. With 500 pounds of limestone, the results are again lower, and turning under the cowpeas and limestone together has not increased the nitric nitrogen above that of the plot on which they were applied separately.

TABLE 12

Average of duplicates in table 10

TREATMENT PER ACRE	NUMBER	FEBRUARY 11	MARCH 1	APRIL 14	MAY 20	JUNE 22
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Control	1	2.6	2.8	5.2	7.3	7.1
2 tons cowpeas	2	4.8	6.5	8.0	10.0	9.7
2000 lbs. $CaCO_3$ applied before 2 tons cowpeas	3	8.9	12.5	11.6	18.2	10.3
2000 lbs. $CaCO_3$ mixed with 2 tons cowpeas	4	6.3	9.4	8.1	14.3	11.1
1000 lbs. $CaCO_3$ applied before 2 tons cowpeas	5	8.6	7.1	5.9	13.6	14.3
1000 lbs. $CaCO_3$ mixed with 2 tons cowpeas	6	7.0	9.0	8.6	20.7	11.4
500 lbs. $CaCO_3$ applied before 2 tons cowpeas	7	9.1	10.0	5.7	14.1	12.3
500 lbs. $CaCO_3$ mixed with 2 tons cowpeas	8	8.5	5.5	9.1	12.1	12.4
2000 lbs. $Ca(OH)_2$ applied before 2 tons cowpeas	9	4.7	1.4	7.8	11.6	8.5
2000 lbs. $Ca(OH)_2$ mixed with 2 tons cowpeas	10	7.0	5.5	7.5	10.9	6.2
500 lbs. $Ca(OH)_2$ applied before 2 tons cowpeas	11	4.0	5.3	9.2	13.7	10.8
500 lbs. $Ca(OH)_2$ mixed with 2 tons cowpeas	12	6.2	7.2	11.5	10.1	10.3
2000 lbs. $CaCO_3$	13	2.7	5.4	7.5	11.5	11.2
1000 lbs. $CaCO_3$	14	4.6	6.4	7.9	13.6	14.5
500 lbs. $CaCO_3$	15	4.2	5.1	7.5	11.6	10.0
2000 lbs. $Ca(OH)_2$	16	4.6	5.2	8.0	13.2	13.7
500 lbs. $Ca(OH)_2$	17	3.7	4.6	6.4	9.0	12.0

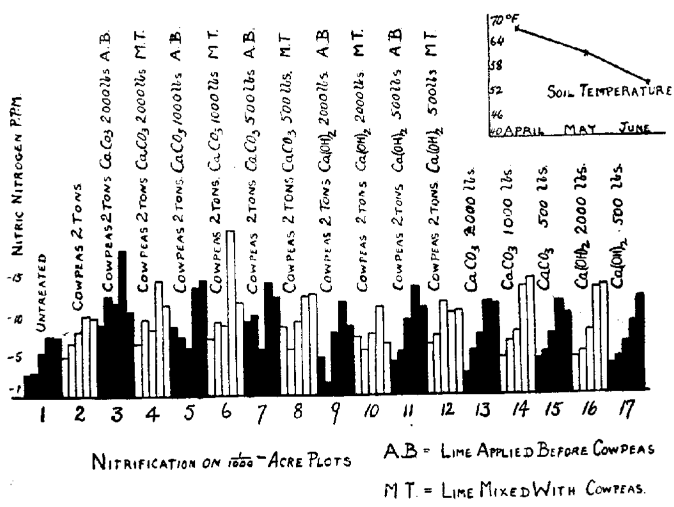

FIG. 9. AMOUNT OF NITRATE PRESENT ON FIVE DIFFERENT DATES FOR EACH TREATMENT

With regard to the manner of application the same holds true concerning 2000 pounds of slaked lime, which in addition seems to have depressed the nitrification, as lower results were obtained than with 500 pounds of limestone.

Five hundred pounds of slaked lime gave better results than 2000 pounds when applied with and before cowpeas, but here again no advantage seems to have been gained by mixing the two together.

When the ground limestone was applied alone the 1000-pound rate gave better results than the 2000 and 500-pound applications. The results were about equal for the application of 2000 pounds and 500 pounds of ground limestone.

When slaked lime at the rate of 2000 pounds was applied alone, bettter results were obtained than by applying it to the same plot as cowpeas. The results with 500 pounds of slaked lime alone, however, are about as good as with the 2000-pound application.

Slaked lime at the rate of 2000 pounds per acre has given slightly better results than the same amount of ground limestone, but with the 500-pound application the limestone is again a little better.

Taking the results throughout, the ground limestone has given somewhat better results than the slaked lime. The limestone at the rate of 1000 pounds per acre has given the best results.

Mixing the cowpeas and lime before turning them under has with the exception of no. 6 (fig. 4) given slightly worse results than when these substances were applied separately. But the slight difference in favor of the separate application would not, in the opinion of the writer, offset the labor saved in turning them under together.

Summary and conclusions for study

From this study the following conclusions seem warranted.

1. Under these conditions of climate green cowpea hay, when turned under in a moist soil, decomposes within two weeks, especially if the operation has been followed by good rains.

This was in extreme contrast to the decomposition of the cowpea hay turned under on the dry-land plots the previous season. The soil was barely moist and no rain worth mentioning fell afterwards. After a prolonged drought of 5 months, traces of cowpeas could still be found in the soil. This shows how carefully the farmer in semi-arid regions must study the weather and soil, in order to get the maximum benefit from turning under green manure. For example, in the instance just mentioned better results were obtained where the cowpeas had been cut for hay, than where they had been turned under, on account of the slow decomposition in a dry soil, which is apt to become drier in the process.

2. Although excessive rains such as were experienced here in February bring about very quick and complete decomposition of the green manure, yet the small increase found in the nitrate subsequently, where the cowpeas

had been turned under, points to the fact that the valuable decomposition products were carried below the first foot of soil. This conclusion is by analogy from the nitrates found deep down on other parts of the farm after these rains.

It would seem that very heavy rains are not desirable on account of this washing down of the soluble products to the lower depths of the soil. Rainfall penetrating about 18 inches would be better, when only the storing of the products of decomposition is considered.

3. The results obtained with slaked lime and ground limestone on a soil slightly alkaline indicate that the ground limestone was the better. This gave the best results at the application of 1000 pounds per acre.

4. The turning in of green cowpea hay and either form of lime together, did not increase the amount of nitric nitrogen as was expected, except in one case. The writer still thinks, however, that the saving of labor will offset any small depression of nitrification which these results seem to indicate.

V. EFFECT OF LIME ON NITRATE PRODUCTION IN NON-IRRIGATED LAND

On January 13, 1920, a virgin strip of land was plowed and cultivated, just beside the strip on which the virgin samples were taken all during the seasonal variation study. On January 15 these $\frac{1}{20}$-acre plots were limed and planted to potatoes at the rate of 1200 pounds per acre. The detailed amounts of the two forms of lime applied will be found in table 13.

The object of the experiment

Many farmers have the idea that lime is necessary for growing potatoes, so the primary object was to obtain data to strengthen the opinion of this station on the matter. As far as the present investigation is concerned, it was an attempt to ascertain how different forms and amounts of lime affected nitrification in non-irrigated soil under field conditions. These seven plots are alongside the potato plots on which the nitrates were determined in the preliminary study. The soil on which the present studies were made is a brown sandy loam which shows a lime requirement of 580 pounds per acre by the Veitch method. It contains 0.06 per cent of nitrogen and has a loss on ignition of 6.3 per cent. At a depth of about 14 inches limonite gravel is found, under plots 1, 2 and 3, but the distance of this from the surface gradually increases and under no. 7 it is nearly 3 feet down.

Before this land was plowed it contained only traces of nitrates; on January 30, 17 days later, 40 part per million were found.

On February 6, when the first samples over all the seven plots were taken, the potatoes were just coming up. On February 24 they were 5 to 6 inches high. On March 4 the plots were cultivated, on the tenth they were ridged up and hand-hoed. By April 9 some were flowering.

The data in table 13 are presented graphically in figure 10.

TABLE 13

Nitrates produced on non-irrigated land with lime

Nitric nitrogen in parts per million, soil moisture in per cent

TREATMENT PER ACRE	PLOT NUMBER	FEBRUARY 6		FEBRUARY 24		APRIL 9		MAY 18		JUNE 17	
		Nitric nitrogen	Soil moisture	Nitric nitrogen	Soil moisture	Nitric nitrogen	Soil moisture	Nitric nitrogen	Soil moisture	Nitric nitrogen	Soil moisture
300 lbs. carbonate of lime	1	10.5	12.3	3.6	15.0	10.5	9.8	5.5	12.0	7.4	10.0
150 lbs. carbonate of lime	2	6.1	12.1	7.5	15.1	1.3	9.4	7.2	10.8	8.6	9.7
1000 lbs. carbonate of lime	3	13.5	10.9	7.1	14.2	4.2	10.2	13.0	10.1	20.2	10.3
300 lbs. slaked lime	4	19.2	12.1	1.4	14.3	1.7	10.3	8.0	10.3	7.1	9.6
150 lbs. slaked lime	5	34.6	11.8	9.4	14.2	2.4	10.3	11.1	9.4	17.8	8.7
1000 lbs. slaked lime	6	22.5	11.5	5.3	13.3	11.5	9.9	10.0	9.0	22.3	8.7
Control	7	16.0	11.4	9.8	13.7	20.7	11.7	23.0	11.1	15.3	11.0

TABLE 14

Nitrates in second foot of plots listed in table 13, February 25, 1920

PLOT NUMBER	NITRIC NITROGEN IN SECOND FOOT	SOIL MOISTURE OF SECOND FOOT	SOIL TEMPERATURE OF PLOTS, JUNE 17
	p.p.m.	*per cent*	°C.
1	2.1	14.2	9.5
2	Trace	14.0	9.7
3	6.8	13.8	9.5
4	12.5	14.6	10.2
5	16.0	15.2	10.0
6	19.1	14.6	12.0
7	14.4	15.0	10.0

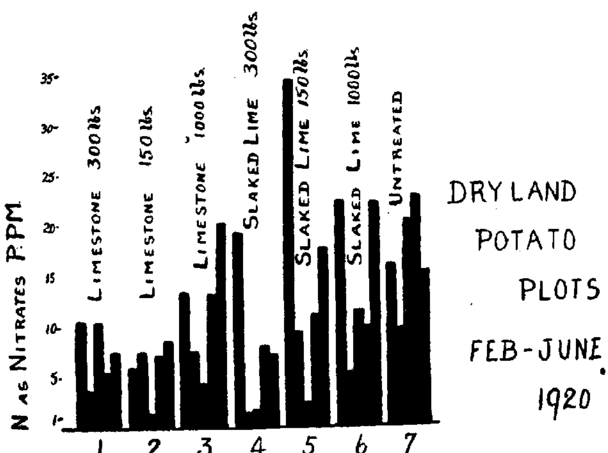

FIG. 10. FIVE DETERMINATIONS OF NITRIC NITROGEN ON POTATO PLOTS BETWEEN FEBRUARY 6 AND JUNE 17, 1920

Discussion of results

With the exception of one plot there was a great deal more nitrogen present in the surface foot of the soil on February 6 than there was on February 24. The rainfall chart (fig. 3) shows that there was over $5\frac{1}{2}$ inches of rain in one week, between those dates. On February 25 the writer obtained second-foot samples on these seven plots, and in four of these deeper samples there was considerably more nitrate than in the surface foot of the previous day.

Although plot 2 on February 24 does not show a drop below its February 6 content, yet its nitrate content on the former date is lower than that of plots 3, 5 and 7, all of which showed a distinct drop at that time.

After the experiment had been started it was found out that long ago a road had crossed this field in the vicinity of plots 1, 2 and 3. In spite of equal plowing and cultivating these three plots seemed to be more compact than the others, as was noticed particularly in taking samples. Plots 1, 2 and 3 are, therefore, probably lower in nitrate content all through on account of bad aeration.

The first four plots are undoubtedly the poorest but of them no. 3, receiving limestone at the rate of 1000 pounds per acre, is decidedly the best.

The high amount of nitric nitrogen in no. 5 on February 6 can hardly be attributed to slaked lime at the rate of 150 pounds per acre, when it is remembered that the check plot on January 30 contained 40 parts per million of nitric nitrogen, which is considerably more.

The untreated plot no. 7 maintains the best nitrate content, considered over the whole period of 5 months. It will also be noticed that it had the best moisture content on the last three determinations.

Comparing the limed plots with the untreated it cannot be found that the liming increased nitrification at all. However, it was noticed during the various samplings that the tilth of plots 5, 6 and 7 was better than that of the first three, and no. 7 appears to be best of all in tilth. This observation corresponds to the nitrate results obtained, and indicates that aeration is a much more important factor for nitrate production than lime, on a newly plowed soil only slightly acid.

Attention has already been drawn to the fact that gravel was found much closer to the surface under plots 1, 2 and 3 than under no. 6 and 7.

In no case is the final nitrate content in June below 7 parts per million. In two cases it is over 20 parts per million and in one over 17 parts per million.

If the nitrate content of the potato land in June, 1920, is compared with that of the teff, cowpea and maize land in the same month, it will be seen that only one of the five contains more than 5 parts per million of nitric nitrogen.

These data are in accordance with the June, 1919, observations recorded in table 1, which shows a much higher nitrate content for the potato plots than for the maize at that time. The actual amount of nitrate, around 5 parts per million, in the maize plots, is very similar for both 1919 and 1920. The amount of nitrate in the potato plots of 1919 is greater than in 1920,

but they were not the same plots for the two years, being located adjacent to each other. The 1919 plots had a better general tilth than the newly plowed and limed plots of the following year, but as the potatoes were dug only on June 28, 1920, these plots did not have the benefit of increased aeration as was the case in 1919.

Attention is again drawn to the rise in nitrates in June over the May amounts, with one exception, although the weather after May 18 became colder and colder, and there were killing frosts, while the soil became drier. The writer has already indicated that he believes this upward rise to be due to partial sterilization of the soil, due to the killing off of the enemies of the nitrifying bacteria by frost. This study seems to indicate the following conclusions.

Conclusions

1. The lower amount of nitric nitrogen on February 24 than was found on the sixth of the month, was due to the very heavy rains between these dates, and the washing down of the nitrates below the first foot. This is quite in accordance with the data obtained at the time on the seasonal-variation plots, and in the depth-sampling study yet to be recorded.
2. The application of two kinds of lime in various amounts did not improve nitrification over the untreated soil.
3. Aeration on this newly cultivated and only slightly acid soil appears to be a much more important factor than lime.
4. The observations made in the 1919 preliminary studies are confirmed in that the potato plots ended up with much more nitric nitrogen than the maize.
5. The potato plots also had more nitric nitrogen in June, 1920, than the teff and cowpea plots in study II.
6. After the cold dry weather and killing frosts of the latter part of May and early June, there is a distinct rise in nitrates in six out of seven plots.

VI. AMOUNTS OF NITRATES IN FIRST FIVE FEET OF POTCHEFSTROOM SOILS

On account of the solubility of nitrates, and the fact that our hot season and rainy season coincide, thus giving conditions for optimum nitrification, it was deemed necessary to take a number of depth samples, to throw some extra light on the observations obtained only from the surface 12 inches.

By the time the first of these samples was taken the middle of the rainy season was upon us, but the evidence is such as to indicate that by January 25 the nitrates had barely passed the third foot.

The samples were taken by digging a pit large enough to enable the operator to handle a pick and reach a depth of 5 feet. One side was kept vertical and clean, and on this face the successive foot-samples were taken.

The results from no. 1 and 2 do not show very high amounts of nitric nitrogen, although both were taken on different fallow strips on the cultivated and irrigated soil which has been studied twice previously.

The results seem to bear out the conclusion in study III concerning the depression of nitrification in irrigated and cultivated soil.

The seepage water collecting in the fourth foot level of no. 1 contained 1.8 parts per million of nitric nitrogen.

As to the amount of nitrogen in the first 4 feet of the fallow strip of the dry-land plots, no. 4 is in great contrast to no. 1 and 2, although the soil-moisture content is not so good on the non-irrigated fallow.

No. 5 and 6 show up worst of all; they are both virgin soils of two very different types, both growing natural veld grass. The low-lying black clay loam showed no nitric nitrogen at all, while the well drained dry-land virgin soil (no. 6) showed just a little in the first foot.

It seems from the seasonal variation study that only a little nitrate is formed under the natural grass, which uses up that little promptly. If no. 7, maize after cowpeas, is compared with the fallow strip no. 4 near by, it will be noted that the average foot content is very similar but that the distribution is very different. It must be remembered, though, that 2.4 inches of rain fell in the last week of January, and this is sufficient to explain the difference in nitrates, in the first foot particularly. It must be noted too that the soil moisture in no. 7 is much greater than in no. 4.

The data furthermore show the benefit of cowpeas over bare fallow as regards nitrification. The strip on which no. 4 was taken had been fallow for 2 years, while no. 7 had grown a crop of cowpea hay, and was growing maize which was over 6 feet high at the time of the sampling, and still its average nitrate content for 5 feet was equal to that of the fallow.

Samples 8 and 9 give the best direct evidence of the effect of heavy rainfall driving the nitrates down into the lower depths, below the feeding zones of the crop roots. Between February 3 and 20 there had been considerable rainfall, including 5.6 inches in one week. Samples 8 and 9 are from the same plot but it will be noticed how much greater is the moisture content from the first to the fifth foot in the samples taken on February 20. Also the nitrates have decreased 75 per cent in the first foot as compared with the sample of February 3, while the nitrates in the third, fourth and fifth feet have increased considerably. In spite of the maize being 17 days older, the total nitrates have increased somewhat if the average content per foot is taken. If no. 8 and 9 are compared with no. 4 and 7 it will be seen that the average nitrate content per foot for 5 feet is better under bare fallow and under maize following cowpeas, than under maize after maize. At the time of sampling, too, the maize on no. 7 was much larger and more vigorous than that on no. 8 and 9. Both the actual yields of maize obtained, and the amounts of nitrates here recorded in table 15, point to the beneficial effects of cowpeas even when cut for hay, instead of being turned under. When

TABLE 15

Amount of nitric nitrogen and soil moisture found at various depths, 1920

DEPTH OF SOIL	NO. 1		NO. 2		NO. 4		NO. 5		NO. 6		NO. 7		NO. 8		NO. 9	
	January 20		January 21		January 23		January 23		January 26		January 30		February 3		February 20	
	Near cage in experiment plots		Near salt-bush in experiment plots		Fallow strip dry-land plots		Virgin soil, black clay loam, low-lying		Virgin soil beside dry-land plots		Maize after cowpeas, dry-land plots		Maize after maize, dry-land plots		Maize after maize, dry-land plots	
	Nitric nitrogen	Soil moisture	Nitric nitrogen	Soil moisture	Nitric nitrogen	Soil moisture	Nitric nitrogen	Soil moisture	Nitric nitrogen	Soil moisture	Nitric nitrogen	Soil moisture	Nitric nitrogen	Soil moisture	Nitric nitrogen	Soil moisture
feet	*p.p.m.*	*per cent*	*p.p.m.*	*per cent*	*p.p.m.*	*per cent*	*p.p.m.*	*per cent*	*p.p.m.*	*per cent*	*p.p.m.*	*per cent*	*p.p.m.*	*per cent*	*p.p.m.*	*per cent*
1	5.8	12.0	4.5	9.5	35.6	8.0	Nil	7.2	0.7	8.7	19.1	11.9	21.1	11.3	5.3	13.0
2	3.5	14.6	Trace	13.5	7.0	10.9	Nil	8.2	Nil	9.5	10.0	13.7	5.2	13.4	5.8	15.5
3	3.7	16.8	Trace	14.6	2.4	12.1			Nil	10.7	6.0	14.2	5.0	14.0	6.3	17.7
4	Nil	17.3	Nil	14.3	1.6	13.3					13.0	14.8	1.2	13.8	12.7	17.3
5											Trace	14.4	Nil	14.2	8.4	18.3
Average nitrate content for 5 feet.............	2.6		0.9		9.3		Nil		0.1		9.6		6.5		7.7	

No. 3 consisted of water which collected overnight by underground seepage in the hole made for sample 1 (Cage). This water contained 1.8 parts per million of nitric nitrogen.

the sample was taken on February 20, the maize was 6 feet high and the ears were just beginning to form.

Some other two feet samples have already been discussed in the previous study. The results shown here with virgin soil together with those recorded in table 15, seem to indicate that nitrates are not found below the first 12 inches in a virgin soil in this locality.

The amounts found in the second foot of the cultivated soils, show that there was considerable nitrate there before the rainy season started, although not as much as was found in the surface 12 inches on the same date (fig. 1 and table 3).

TABLE 16

Nitrates and soil moisture on various second-foot samples

LOCALITY	REMARKS	DATE OF SAMPLING	NITRIC NITROGEN	SOIL MOISTURE
			p.p.m.	*per cent*
Virgin soil	From the non-irrigated plots studied for seasonal variation	Oct. 3, 1919	Nil	9.8
Teff	From the non-irrigated plots studied for seasonal variation	Oct. 3, 1919	1.8	10.9
Cowpeas harvested	From the non-irrigated plots studied for seasonal variation	Oct. 3, 1919	5.1	12.8
Cowpeas plowed under	From the non-irrigated plots studied for seasonal variation	Oct. 3, 1919	4.7	13.4
Fallow	From the non-irrigated plots studied for seasonal variation	Oct. 3, 1919	6.8	13.6
Virgin soil	From the non-irrigated plots studied for seasonal variation	Oct. 8, 1919	Nil	9.3
Cultivated soil	From irrigated land used in study III	Oct. 8, 1919	1.8	8.2
Virgin soil	From southeast of football field, same as used in study III	Oct. 8, 1919	Nil	6.6

If the amounts of nitrate found in these soils below the first foot are compared with those recorded by other workers, it can be seen that they are much superior to amounts recorded by Stewart in the Cache Valley, Utah, and also superior to the nitrate content of most of the depth samples taken at the Longerenong Agricultural College, Victoria, Australia, by Scott, Robertson and Richardson (36). Both these stations receive several inches less rainfall than Potchefstroom. The amounts of nitrate that Buckman found in 5-foot samples in Montana (5), however, are much higher than these recorded in this study by the writer. Buckman's figures were also obtained under a lower rainfall than that prevailing here.

On the other hand, the nitrates at different depths of the non-fertilized cultivated soil on this station are higher than those which the writer has seen recorded at the Rothamsted station.

Summary of study VI

1. A comparison of the nitric nitrogen in the first 5 feet of a non-irrigated cultivated soil with that in an irrigated cultivated soil, shows the non-irrigated soil of the same type to be much superior in nitrate content, although the irrigated soil does not get sufficient water to carry the nitrates to the fourth foot.

2. The virgin soil of this station shows very little nitrate in the surface 12 inches, and so far none has been recorded below this depth.

3. Land with a 6½-foot-high maize crop following cowpeas cut for hay, shows as good a nitrate content for 5 feet as the same land which has been bare-fallowed during that time.

4. Although the nitrates during a very active growing period of the maize between February 3 and 20 have decreased 75 per cent in the surface foot, yet to a 5-foot depth they have increased. The decrease in the surface foot is attributed chiefly to the heavy rainfall.

5. Land bare-fallowed and land on which maize followed cowpeas cut for hay, had a better nitrate content than the same land on which maize followed maize.

VII. THE NITRIFYING POWER OF SOIL AT DIFFERENT DEPTHS

Apart from gathering some data concerning the amounts of nitrate found in the soil per foot in 5-foot sections, it was thought advisable to determine the actual nitrifying power of the soil at different depths.

For this purpose on April 12, 1920, a 5-foot hole was dug in the "maize after maize" section of the dry-land plots. One side of the hole was cut quite vertical and from this the successive foot-sections were cut and placed in sterilized glass screw-top jars. The spade was thoroughly washed and scrubbed and then immersed in strong lysol, after which it was again rinsed with clean tap-water, every time it was used. The surface and sides of each section were shaved with a clean spade, which was again cleansed as described above before the sample was taken. A sheet of zinc cleaned in the same way as the spade was used for catching each sample.

Each foot-sample was placed on clean brown paper and allowed to become thoroughly air-dried. One-hundred-gram portions were weighed off into small tins, and mixed with 0.07 gm. of dried blood, 0.03 gm. of ammonium sulfate, 0.17 gm. of bone meal, and 0.17 gm. of dried and finely ground cowpea hay. These amounts were sufficient to supply each 100 gm. of air-dry soil with 7.0 mgm. of nitrogen on which to test its nitrifying power.

The moisture content was made up to 70 per cent of the soil's water-holding capacity, and the tins were placed on a shelf of a store-room and lightly covered with brown paper on April 21. The writer had no incubator at his disposal, so the tins were left at room temperature for 6 weeks in a building not heated in any way. Meanwhile, the weather gradually became colder, as will be

seen from the temperature chart. This small room, however, has a very even temperature and for the first 2 weeks the thermometer remained in the vicinity of 65°F., but then it declined steadily, and during the last two weeks of the six, it ranged from 40° to 55°F. The temperature during the period of incubation was not sufficiently low to supress nitrification, but it undoubtedly retarded it. Table 17 records the nitrifying power of the soil at different depths.

TABLE 17

Nitrifying power of soil at different depths

DEPTH OF SOIL	SOIL ALONE			SOIL AND DRIED BLOOD		SOIL AND BONE MEAL		SOIL AND AMMONIUM SULFATE		SOIL AND COWPEA HAY	
	Nitrate at start	Nitrate after 6 weeks	Increase or decrease	Total nitric nitrogen	Increase or decrease from dried blood	Total nitric nitrogen	Increase or decrease from bone meal	Total nitric nitrogen	Increase or decrease from ammonium sulfate	Total nitric nitrogen	Increase or decrease from cowpea hay
feet	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
1	8.5	15.9	7.4	13.2	−2.7	22.5	6.6	17.2	1.3	20.5	4.6
2	19.3	19.8	0.3	24.2	4.4	22.0	2.2	23.4	3.6	22.6	2.8
3	9.3	11.4	2.1	9.4	−2.0	9.5	−1.9	10.0	−1.4	11.7	0.3
4	1.2	1.5	0.3	Nil	−1.5	Nil	−1.5	0.9	−0.6	0.6	−0.9
5	1.1	0.6	−0.5	0.5	−0.1	Nil	−0.6	Nil	−0.6	Nil	−0.6

The temperatures of the depth samples are included in the table 18 together with other data.

TABLE 18

Data supplementary to table 17

SOIL TYPE AND REMARKS	DEPTH OF SOIL	LOSS ON IGNITION	NITROGEN	VEITCH LIME REQUIREMENT	HYGROSCOPIC MOISTURE	TEMPERATURE ON APRIL 12, 1920
	feet	*per cent*	*per cent*		*per cent*	°C.
Brown fine sandy loam containing an appreciable amount of clay; samples taken on dry-land plots, "maize after maize" section	1	6.7	0.09	Slightly alkaline	1.9	21.2
	2	7.6	0.04	Slightly alkaline	1.9	21.0
	3	8.3	0.06	Slightly alkaline	2.4	20.8
	4	9.0	0.05	Slightly alkaline	2.6	20.0
	5	8.8	0.05	Slightly alkaline	3.4	20.0

This soil begins to get gravelly in the third foot, while the fourth and fifth feet consist of 80 per cent of concretionary limonite gravel not passing a 3-mm. sieve. The higher loss on ignition in the lower feet is probably due to the loss of water of hydration, the light yellow color of the soil indicating much less organic matter than in the first 2 feet. The amount of hygroscopic moisture also increases with successive depths.

In spite of unfavorable conditions there was considerable nitrification in the first foot, as will be seen from table 17, also some activity in the second foot,

a little in the third foot, but a loss in nitrates in every case in the fourth and fifth feet, where nitrogenous fertilizers have been added. Beckwith, Vass and Robinson (2) in Oregon also found that dried blood and ammonium sulfate sometimes depressed nitrification. The total amount of nitric nitrogen in the second foot is greater, but it was greater at the beginning, and has not increased as much during incubation as that in the first foot.

A number of other soils which will be recorded in the next study, were incubating under the same conditions as the above, and as some high amounts of nitrate were obtained in these the temperature could not have been very much against nitrification. The results in any case will show the relative nitrifying power of the different foot sections.

The third foot shows some little nitrification of the soil nitrogen, but a loss wherever dried blood, bone meal and ammonium sulfate have been added.

In general, bone meal has been best nitrified, followed in order by cowpea hay, ammonium sulfate, and dried blood.

Lipman (20) obtained nitrification in a number of samples from the fifth foot and even lower depths of California soil when incubated. Kelly (19) also shows strong nitrification by the fifth foot of a soil he studied in that region, while McBeth and Smith (33) with soils from the Greenville Experiment Farm, Utah, obtained much better results than the writer's, especially in the third, fourth and fifth feet. The bad, gravelly limonite subsoil (80 per cent gravel), may account in some way for the poor nitrification in the Potchefstroom third, fourth and fifth feet.

The writer intends to carry on this work with depth samples from other soil types in this vicinity.

Summary

1. In this particular soil type there appears to be hardly any nitrification below the second foot.
2. This seems further evidence that the large amount of nitric nitrogen, obtained in the fourth and fifth feet of several samples taken from this vicinity, and reported in the last study, was brought there from the first and second feet by rain-water.
3. The addition of nitrogenous fertilizers to the third, fourth and fifth-foot samples depressed nitrification.
4. There is a gradual decrease in the total nitrogen content of the soil from the first to the fourth foot.
5. The loss on ignition, on the other hand, shows an increase with depth which does not correspond to the decreasing amount of total nitrogen, as is usually the case, but does correspond with the increase in hygroscopic moisture.

It is likely there is considerable loss of water of hydration from the limonite, which would also account for the increase of loss on ignition of the successive feet.

VIII. A NITRIFICATION STUDY ON WHALE MANURE

About 1500 tons of fertilizer are produced yearly in South Africa as a by-product of the whaling industry (32). After the blubber has been extracted the whale carcass is worked up into a fertilizer, in much the same way as meat meal from abattoirs. This product is sold under the name "whale guano."

A fertilizer manufacturer gave the writer three differently treated whale guanos: (a) ordinary whale guano, (b) ether-extracted whale guano and (c) sulfated whale guano.

An endeavor was made to ascertain whether the ether-extracted guano did not nitrify better than the ordinary whale guano, on account of being quite free of fat and oil. The ether extract amounted to 14.9 per cent of the original whale guano. The second point aimed at was whether the percentage of nitrification of the sulfated guano was not better than that of the ordinary, untreated substance. The sulfated guano contained 9.1 per cent of P_2O_5, of which 7.53 per cent was water-soluble.

A fine brown sandy loam, with a lime requirement of 1000 pounds per acre by the Veitch method, was used. One gram of each of the guanos was mixed with 100 gm. of air-dry soil, and distilled water was added to make up 70 per cent of the soil's water-holding capacity. The soils were incubated for 30 days at 28 to 30°C., and then the nitric nitrogen was determined.

The results obtained and the amounts of nitrogen in the soil and the guano are given separately in table 19.

TABLE 19

Nitrification of whale guanos

SUBSTANCE USED	NITROGEN IN SOIL ALONE AND IN WHALE GUANO ALONE	TOTAL NITRATE PRODUCED	INCREASE OF NITRATE OVER SOIL ALONE	SOIL NITROGEN NITRIFIED AND GUANO NITROGEN NITRIFIED
	per cent	*p.p.m.*	*p.p.m.*	*per cent*
Soil alone	0.069	75.6		10.95
Soil and ordinary whale guano	7.972	139.8	64.2	8.05
Soil and ether-extracted whale guano	8.700	153.2	77.6	8.98
Soil and sulfate whale guano	3.187	122.9	47.3	14.84

According to this test, the nitrogen of the sulfated whale guano was the most efficiently nitrified, while the differences between the percentages of the ordinary whale guano and the ether-extracted falls well within the experimental error. The substances removed in the ether extract do not appear to retard nitrification. Taking equal weights of the sulfated and untreated whale guano, it can be seen that the sulfated contains 4.79 per cent less nitrogen, or 95.8 pounds less per ton of 2000 pounds. In this country nitrogen is worth about $1^s/1^d$ per pound, so the loss approximates 104^s per ton. In the writer's opinion this loss is not compensated by making 7.5 per cent of phos-

phoric oxide water-soluble or in making the remaining nitrogen more easily nitrifiable within a month.

In the study following this it will be seen that whale guanos were again used on two different soils of this district, in a comparative study of the nitrifiability of various nitrogeneous fertilizers. In the one soil, a reddish brown sandy loam of a heavier type than the one used in this study, and of about 600 pounds lime requirement, the sulfated guano is still ahead, but in the black clay loam containing 5.93 per cent of carbonate of lime, the sulfated guano produced the least nitrate of the three, whereas the ordinary whale guano in this soil gave quite the best results of the three, in fact was among the most efficiently nitrified of the nitrogenous fertilizers.

Summary

1. In two out of three soils, both of which were slightly acid, the sulfated guano was best nitrified.
2. Although rendering water-soluble a small amount of phosphate and sometimes giving a better nitrifiable product, it is doubtful whether the sulfating process is advisable, in view of the fact that it causes a loss of nearly 5 per cent of total nitrogen.
3. The 14.9 per cent of material which can be extracted from ordinary whale gunao by ether does not appear to suppress nitrification, as was popularly supposed.

IX. A COMPARATIVE STUDY OF THE NITRIFIABILITY OF TEN NITROGENOUS FERTILIZERS

The fertilizers used in this study were three differently treated whale guanos, calcium cyanamide, crayferine, which is the dried and ground by-product of the crayfish canning industry, dried blood, bone meal, ammonium sulfate, dried and finely ground cowpea hay and soil treated with sewage sludge.

The two most important soil types of this district were chosen for this test. The one soil is a reddish-brown loam, having a lime requirement of about 600 pounds per acre by the Veitch method. The sample was taken from the fallow strip of our dry-land fertilizer plots. This soil is representative of the chief dry-land type on which most of the maize and teff grass in this district are grown.

The other soil is a black clay loam containing 5.93 per cent of free carbonate of lime. It is of alluvial origin and is found chiefly for three to four hundred yards on both banks of the Mooi River and its tributaries. It is the chief soil type under irrigation in these parts, and is one of the richest soils both chemically and practically that the writer has met with in South Africa.

The field where this particular sample was taken has been under cultivation for 35 years. It has grown lucerne rye, oats, mangels and Sudan grass, and in dry seasons sometimes maize and teff grass.

According to Lipman and Burgess (23) the testing of nitrogenous fertilizers for availability is better done by nitrification than by ammonification trials, as many soils which produce ammonia readily do not produce much nitrate. According to many investigators, although crops do take up their nitrogen in various forms, it is taken up chiefly as nitrates. This is a further reason for making a nitrification instead of an ammonification test, to ascertain the relative value of these ten nitrogenous fertilizers on our two main soil types.

Apart from the above object, the writer wished to find out whether small quantities of the fertilizer, more in accordance with actual practice, were not better nitrified than large amounts in these soils. This knowledge would help in the ultimate decision of the amounts which should be used in such trials, a matter that is receiving considerable attention.

The writer also had in mind the opinions of Lohnis and Green (26), Allen and Bonazzi (1), and Kelly (19) on this same matter. All these workers show clearly the error in using large amounts of fertilizer for this test and point out that the value of the work of many investigators has been vitiated on this account.

Green, in his later work on nitrogen metabolism, used only enough ammonium sulfate to add 11.2 mgm. of nitrogen to each test flask (10).

The writer is particularly interested in the publications of the workers on semi-arid soils. Lipman and Burgess in one of their studies on California soils used 1 gm. of each fertilizer irrespective of the precentage of nitrogen it contained (21). Later Lipman, Burgess and Klein (24), in an incubation nitrification experiment on a great number of humid and arid soils, used 1 gm. each of cottonseed meal and dried blood and 0.2 gm. of ammonium sulfate. These supplied 47, 132 and 40 mgm. of nitrogen, respectively, to each 100 gm. of soil. It is noteworthy that the arid soils nitrified best the cottonseed meal and ammonium sulfate, both of which contained around 40 mgm. of nitrogen, while the blood meal containing three times that amount of nitrogen was not so well nitrified. The soils from humid areas, however, produced a higher percentage of nitrate from dried blood than the substances containing less nitrogen. Lipman and his co-workers state that they are well aware that 0.05 and 0.1 gm. of dried blood might have been better nitrified than 1 gm., but they believe that as the humid and arid soils were treated alike their results clearly give relative and comparative nitrification figures, although they may not be absolute values.

Kelly (19), also working on California soils, found that portions of dried blood containing 0.125 and 0.0625 gm. of nitrogen were quite as well nitrified as bone meal and ammonium sulfate containing approximately equal quantities of nitrogen. He points out that dried blood gives good results under field conditions in California when applied up to the rate of 1080 pounds per acre and is well nitrified. He concludes that Lipman's bad results with dried-blood nitrification trials on California soils is due to his using 18.5 times more than is applied in actual farm practice.

Sakett in Colorado (40) used 100 mgm. of nitrogen in the form of ammonium sulfate, ammonium carbonate and ammonium chloride. Fraps (8) in Texas used 50 mgm. of nitrogen in the form of sheep manure and ammonium sulfate to test the nitrifying power of the soils of that state.

The writer in this study used 1 gm. of each fertilizer irrespective of its nitrogen content and also just enough to add 7 mgm. of nitrogen to each 100 gm. of soil. This is a lower amount than was used by any of the workers herein referred to, except Brenchley and Richards (3) who used 6 mgm. of nitrogen as sewage sludge. When worked out on the acre basis 7 mgm. would give 175 pounds of nitrogen per acre, or 1750 pounds of a 10 per cent dried blood. This is more than ten times the amount usually applied under field conditions in South Africa, but it is doubtful whether smaller quantities of material could be used without introducing a very large experimental error.

The writer has chosen the incubation time and temperature as used by the majority of workers on semi-arid soils, viz., one month (30 days) at 28° to 30°C. At the beginning the soils had distilled water added up to 70 per cent of their water-holding capacity, and on two other occasions small quantities were added to make up for what was lost in the incubator. The results are given in table 20.

If the sewage soil and calcium cyanamide results are omitted, then the remainder show that in 62.5 per cent of the results, the smaller amount of nitrogen was more efficiently nitrified than the larger, under the conditions of the experiment. One gram of ammonium sulfate was fairly well nitrified in the black clay loam containing plenty of lime, but in the other slightly acid soil, nitrification was depressed very much.

With the two exceptions already named, both soils nitrified the other fertilizers quite well, although the black clay loam produced considerably better results than the other. Crayferine, ammonium sulfate, ordinary whale guano, and cowpea hay were best nitrified.

The soil treated with sewage sludge, strange to say, gave very unexpected results, and probably the sample sent here contained some substance deleterious to nitrification.

Both Lipman and Burgess (22) and Brenchley and Richards (3) report very favourable nitrification results with sewage sludge, in California and in England.

The writer was sent a sample supposed to be sewage sludge, but on analysis it had only 0.33 per cent of nitrogen, and on inquiry was found to be soil treated with sewage sludge and not the sludge itself. The original sample contained, in addition to the above nitrogen, 465.4 parts per million of nitric nitrogen.

Calcium cyanamide, in both small and large amounts, depressed nitrification below that of the soil control. Some field trials give results which make this substance compare favorably with sodium nitrate and ammonium sul-

TABLE 20

Comparative nitrification of ten fertilizers

SOIL AND FERTILIZERS	SOIL 53, BLACK CLAY LOAM				SOIL 54, BROWN SANDY LOAM			
	Nitrogen added by fertilizer	Total nitrate found	Increase over soil nitrate alone	Nitrogen nitrified	Nitrogen added by fertilizer	Total nitrate found	Increase over soil nitrate alone	Nitrogen nitrified
	mgm.	*p.p.m.*	*p.p.m.*	*per cent*	*mgm.*	*p.p.m.*	*p.p.m.*	*per cent*
Soil alone	157.0	64.6		2.0	100.0	71.8		5.14
Soil and ordinary whale guano	79.72	414.4	349.8	43.9	79.72	208.6	136.8	17.1
Soil and ordinary whale guano	7.0	136.0	71.4	102.0	7.0	48.5	Loss	Nil
Soil and ether-extracted whale guano	87.0	438.8	374.2	43.0	87.0	325.6	153.8	17.6
Soil and ether-extracted whale guano	7.0	76.5	11.9	17.0	7.0	80.9	9.1	13.0
Soil and sulfated whale guano	31.87	170.4	105.8	33.1	31.87	139.0	67.2	21.1
Soil and sulfated whale guano	7.0	65.7	1.1	1.6	7.0	93.7	21.9	31.2
Soil and calcium cyanamide	180.0	4.3	Loss	Nil	180.0	1.3	Loss	Nil
Soil and calcium cyanamide	7.0	19.0	Loss	Nil	7.0	32.9	Loss	Nil
Soil and crayferine	60.8	274.0	209.4	34.4	60.8	307.3	235.5	38.7
Soil and crayferine	7.0	139.8	75.2	107.4	7.0	128.1	56.3	80.4
Soil and dried blood	105.0	666.4	601.8	57.3	105.0	84.6	12.8	1.2
Soil and dried blood	7.0	109.7	45.1	64.2	7.0	79.4	7.6	10.8
Soil and bone meal	40.0	240.5	175.9	43.9	40.0	264.1	192.3	48.1
Soil and bone meal	7.0	97.6	33.0	47.1	7.0	42.4	Loss	Nil
Soil and ammonium sulfate	205.0	771.1	706.5	34.4	205.0	1.6	Loss	Nil
Soil and ammonium sulfate	7.0	122.6	58.0	82.8	7.0	114.5	42.7	61.0
Soil and ammonium sulfate	41.0	503.6	439.0	107.0	41.0	151.6	79.8	19.4
Soil and cowpea hay (dried)	40.1	230.2	165.6	41.3	40.1	189.8	118.0	29.4
Soil and cowpea hay (dried)	7.0	105.3	40.7	58.1	7.0	92.0	20.2	28.9
Soil and sewage soil	3.3	54.5	Loss	Nil	3.3	30.7	Loss	Nil
Soil and sewage soil	1.9	39.6	Loss	Nil	1.9	56.9	Loss	Nil

Soil 53 contained 33.0 parts per million of nitric nitrogen before incubation.
Soil 54 contained 20.4 parts per million of nitric nitrogen before incubation.

fate; others show it to be harmful. The writer's results are quite in accord with those of Lipman and Burgess (21) with calcium cyanamide on semi-arid soils. These American workers point out that the European investigators, Grazia, Muntz, and Nottin, noted that if much longer incubation periods were allowed, the nitrogen in this fertilizer was finally almost completely transformed into nitrate.

This substance is not readily obtainable on South African markets and it would seem wiser for farmers to leave this form of nitrogenous fertilizer alone until the time it takes to be nitrified in our soils has been more accurately determined, as planting too soon after applying this fertilizer almost always affects the germinating seed adversely.

Summary

1. This nitrification study showed that all the fertilizers with two exceptions were well nitrified.

2. The crayferine was best nitrified in all cases, followed in order by ammonium sulfate, ordinary whale guano, cowpea hay, bone meal, dried blood, ether-extracted whale guano, and sulfated whale guano.

3. With the exception of the sulfated whale guano, the fertilizers were much better nitrified in the alkaline black clay loam than in the slightly acid brown sandy loam.

4. The soil nitrogen in the lighter soil, however, was much more efficiently nitrified than that of the heavier type.

5. In 62.5 per cent of the cases, the smaller amount of nitrogen was more efficiently nitrified than the larger.

6. These results would seem to be an additional argument in favor of using smaller quantities of material, more in accordance with field practice, for nitrification studies such as these.

X. THE NITRIFYING POWERS OF VARIOUS SOUTH AFRICAN SOILS[2]

On three separate occasions, between April and September, the writer received batches of soils from many sections of the Union for this study. The first batch of 18 samples were taken by the chemists or officers in charge of our different experiment stations in each province.

These soils had finished their 6 weeks' incubation by June. The other two batches arrived in August and September, and were taken by farmers who had attended the June Short Course and who had, after hearing what care had to be exercised in taking and despatching the samples, volunteered to undertake the work. Apart from careful personal instruction in the manner of sampling, the writer sent detailed typewritten directions to each of the

[2] The writer wishes to thank his colleagues, and the farmers who so kindly took and forwarded these soil samples from all over the Union, and so made this investigation possible.

TABLE 21

The nitrifying powers of various soils—nitric nitrogen

NUMBER	LOCALITY AND SOIL TYPE	SOIL ALONE				SOIL AND DRIED BLOOD		SOIL AND BONE MEAL		SOIL AND AMMONIUM SULFATE		SOIL AND DRIED COWPEA HAY	
		At start	At finish	Increase	Nitrogen nitrified	Total	Increase or decrease from dried blood	Total	Increase or decrease from bone meal	Total	Increase or decrease from $(NH_4)_2SO_4$	Total	Increase or decrease from cowpea hay
		p.p.m.	*p.p.m.*	*p.p.m.*	*per cent*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
1	Rustenburg virgin sandy loam	0.8	0.9	0.1	0.02	0.6	−0.3	0.9		1.2	0.3	0.8	−0.1
2	Rustenburg cultivated sandy loam	27.6	65.7	38.1	5.08	73.2	7.5	83.7	18.0	93.2	27.5	53.7	−12.0
3	Glen O. F. S. virgin clay loam	34.5	51.3	16.8	1.57	43.9	−7.4	37.2	−14.1	45.6	−5.7	31.5	−19.8
4	Glen O. F. S. cultivated clay loam	0.7	11.6	10.9	1.29	31.5	19.9	26.0	14.4	21.5	9.9	17.2	5.6
5	Glen O. F. S. virgin fine sandy loam	0.7	10.1	9.4	2.68	4.8	−5.3	9.5	−0.6	8.7	−1.4	6.1	−4.0
6	Glen O. F. S. cultivated fine sandy loam	1.0	5.9	4.9	0.9	11.0	10.0	17.9	12.0	19.1	13.2	7.4	1.5
7	Cedara virgin silt loam	0.6	2.5	1.9	0.08	1.6	−0.9	1.7	−0.8	1.4	−1.1	2.7	0.2
8	Cedara cultivated silt loam	7.4	31.2	23.8	0.95	48.7	17.5	56.0	24.8	40.4	9.2	56.0	24.8
9	Winkle spruit virgin fine sand	2.0	8.3	6.3	2.73	23.7	15.4	17.2	8.9	2.5	−5.8	18.6	10.3
10	Winkle spruit cultivated fine sand	0.8	0.6	−0.2	Nil	1.2	0.6	1.7	1.1	1.0	0.4	1.0	0.4
11	Winkle spruit virgin fine sand	1.7	2.2	0.5	0.1	0.9	−1.3	0.7	−1.5	1.4	−0.8	1.6	−0.6
12	Winkle spruit cultivated fine sand	1.1	1.5	0.4	0.12	0.6	−0.9	0.6	−0.9	0.6	−0.9	0.7	−0.8
13	Elsenburg virgin loam	0.9	3.5	2.6	0.4	6.2	2.7	8.5	5.0	1.1	−2.4	4.6	1.1
14	Elsenburg cultivated loam	2.2	1.3	−0.9	Nil	5.0	3.7	4.5	3.2	0.8	−0.5	2.6	1.3
15	Middelburg virgin loam	1.0	8.0	7.0	0.68	28.9	20.9	32.5	24.5	30.2	22.2	19.7	11.7
16	Middelburg cultivated loam	0.9	12.7	11.8	1.3	85.0	72.3	72.3	59.6	75.9	63.2	31.5	18.8
17	Middelburg virgin loam	1.0	11.0	10.0	0.95	48.0	37.0	33.8	22.8	34.5	23.5	17.3	6.3
18	Middelburg cultivated loam	5.4	30.9	25.5	2.34	117.9	87.0	43.5	12.6	87.7	56.8	58.0	27.1
19	Klerksdorp virgin sandy loam	2.9	40.4	37.5	4.31	74.5	34.1	94.6	45.2	89.5	49.1	70.6	30.2
20	Klerksdorp cultivated sandy loam	7.1	15.4	8.3	1.06	112.1	96.7	46.4	31.0	64.1	48.7	44.5	29.1
21	Rustenburg virgin sandy loam	0.8	12.0	11.2	2.6	3.2	−8.8	12.9	0.9	9.8	−2.2	18.0	6.0
22	Rustenburg cultivated sandy loam	0.8	Trace	−0.8	Nil	4.7	4.7	24.6	24.6	3.2	3.2	7.7	7.7
23	Kuruman virgin fine sandy loam	0.8	25.1	24.3	2.53	73.1	48.0	30.0	4.9	58.2	33.1	45.9	20.8
24	Kuruman cultivated fine sandy loam	1.3	53.6	52.3	3.84	100.9	47.3	77.3	23.7	79.1	25.5	55.5	1.9

25	Standerton virgin clay loam	1.0	10.0	9.0	0.44	5.0	−5.0	14.6	4.6	4.7	−5.3	8.5	−1.5
26	Standerton cultivated clay loam	7.2	84.1	76.9	4.99	82.7	−1.4	74.1	−10.0	77.5	−6.6	59.9	−24.2
27	Krugersdorp virgin sandy loam	0.1	10.8	10.7	1.81	8.2	−2.6	56.0	45.2	18.4	7.6	23.2	12.4
28	Krugersdorp cultivated sandy loam	2.5	21.2	18.7	3.46	69.1	47.9	54.2	33.0	39.3	18.1	45.6	24.4
29	Pietersburg virgin coarse sand	0.7	14.3	13.6	2.61	65.7	51.4	56.1	41.8	65.0	50.7	21.0	6.7
30	Pietersburg cultivated coarse sand	1.3	8.5	7.2	1.5	16.1	7.6	17.6	9.1	75.8	67.3	25.8	17.3
31	Ventersdorp virgin dark chocolate loam	0.6	7.2	6.6	0.8	44.0	36.8	67.7	60.5	45.2	38.0	24.8	17.6
32	Ventersdorp cultivated dark chocolate loam	3.7	21.9	18.2	2.84	60.2	38.3	24.4	2.5	21.9		51.6	29.7
33	Koekemoer virgin red sandy loam	0.6	14.6	14.0	2.29	11.8	−2.8	35.0	20.4	45.4	30.8	16.9	2.3
34	Koekemoer cultivated red sandy loam	4.8	17.5	12.7	2.64	66.2	48.7	24.4	6.9	17.6	0.1	26.9	9.4
35	Lichtenburg virgin brown sandy loam	0.7	2.5	1.8	2.14	33.5	31.0	35.5	33.0	43.2	40.7	23.7	21.2
36	Lichtenburg cultivated brown sandy loam	2.3	25.1	22.8	3.16	76.8	51.7	55.2	30.1	57.4	32.0	30.2	5.1
37	Ladybrand virgin fine sandy loam	0.6	2.0	1.4	0.25	17.3	15.3	17.6	15.6	7.7	5.7	3.4	1.4
38	Ladybrand cultivated fine sandy loam	4.3	26.3	22.0	3.18	81.5	55.2	64.9	38.6	37.1	10.8	73.5	47.2
39	Kliptown virgin black clay loam	2.8	175.2	172.4	2.25	200.2	25.0	196.0	20.8	282.2	107.0	36.1	−139.1
40	Rosetta Natal virgin light brown silt loam	2.6	39.0	36.4	1.01	68.2	29.2	107.0	68.0	71.0	32.0	35.5	−3.5
41	Rosetta Natal cultivated light brown silt loam	15.6	63.0	47.4	1.56	35.2	−27.8	103.0	40.0	87.9	24.9	13.8	−49.2
42	Marquard virgin light brown fine sandy loam	0.8	15.4	14.6	2.4	54.0	38.6	39.0	23.6	16.1	0.7	6.0	−9.4
43	Marquard cultivated light brown fine sandy loam	6.3	21.7	15.4	2.65	61.4	39.7	27.6	5.9	62.6	40.9	23.7	2.0
44	Butterworth virgin grey sandy loam	1.0	22.9	21.9	2.38	47.1	24.2	70.3	47.4	45.4	22.5	50.0	27.1
45	Butterworth cultivated grey sandy loam	2.7	21.3	18.6	2.75	64.1	42.8	54.2	32.9	45.9	24.6	18.5	−2.8
46	Melmoth Zululand virgin brown loam	0.8	5.7	4.9	0.3	5.1	−0.6	10.6	4.9	3.6	−2.1	4.1	−1.6
47	Melmoth Zululand cultivated brown loam	5.3	75.3	70.0	0.4	119.5	44.2	106.8	31.5	66.2	−9.1	53.0	−22.3
48	Mooi River Natal virgin light brown clayey loam	0.9	Trace	Loss	Nil	Trace	Loss	Trace	Loss	Trace	Loss	Trace	Loss
49	Mooi River Natal cultivated light brown clayey loam	1.3	32.4	31.1	3.3	59.3	26.9	50.6	18.2	61.8	29.4	4.7	−27.7
50	Molteno virgin light brown sandy loam	1.9	6.2	4.3	0.5	40.4	34.2	50.3	44.1	48.7	42.5	5.1	−1.1
51	Molteno cultivated dark brown clay loam	14.6	70.7	56.1	4.0	60.7	−10.0	97.2	26.5	115.1	44.4	97.5	26.8
52	Potchefstroom virgin greyish black clay loam	5.9	65.7	59.8	3.0	129.1	63.4	126.1	60.4	166.3	100.6	80.0	14.3
53	Potchefstroom cultivated greyish black clay loam	33.0	64.6	31.6	2.0	109.7	45.1	97.6	33.0	122.6	58.0	105.3	40.7
54	Potchefstroom cultivated brown sandy loam	20.4	71.8	51.4	5.14	79.4	7.6	42.4	−29.4	114.5	42.7	92.0	20.2

volunteer farmers when he was ready for the soils. From the keen interest shown, there is every reason to believe that the samples were carefully taken.

The samples on arrival, were all spread out on clean brown paper and thoroughly air-dried before being passed through a 3-mm. sieve, and weighed out into 100-gm. portions.

Soils 1 to 18 were incubated at room temperature for 6 weeks, as described in study VII. Soils 19 to 39 were incubated for 30 days at 28 to 30°C., and when they were completed, no. 40 to 54, which arrived later, were treated in like manner. It is to be regretted that no incubator was available when no. 1 to 18 arrived, but it is hoped that the 2 weeks' longer time has largely counterbalanced the lower temperature. The figures are not quite comparable, although the amount of soil nitrogen nitrified in several soils of this first batch is as high as the best results obtained with the batches in the incubator at a higher temperature.

The time and temperature of the incubation period were specially chosen, and they were the same as were used by Lipman and his co-workers in their comparative study on the nitrifying powers of 150 arid and 45 humid soils.

The writer bore the fact in mind that in this manner he would be able to obtain data which would be directly comparable with those on other semi-arid soils, and also with the results on humid soils on another continent. In this manner, he would be able to obtain further evidence to show whether or not nitrification in South African soils is as intense as has been supposed and reported in the past.

Lipman, Burgess and Klein used 1 gm. of dried blood, containing 132 mgm. of nitrogen, 1 gm. of cottenseed meal, containing 47 mgm. of nitrogen, and 0.2 gm. of ammonium sulfate, giving 40 mgm. of nitrogen.

The writer used enough dried blood (10.5 per cent nitrogen), ammonium sulfate (20.5 per cent nitrogen), bone meal (4 per cent nitrogen), and dried and finely ground cowpea hay (4 per cent nitrogen), to give to each 100 gm. of air-dry soil 7 mgm. of nitrogen. The reason for this small amount has been fully discussed in the previous study, which also showed that the percentage of nitrification is apt to be higher on account of using a smaller quantity of the fertilizer. If, then, the California soils and those from humid regions prove superior, it will not be for want of suitable conditions for the South African soils.

The best basis for comparison, however, will be the nitrification of the soil nitrogen itself, as that, after all, is the most important substance. The nitrification of the fertilizers will be discussed, but for the ultimate comparison of nitrification in those soils with soils of other countries, the percentage of soil nitrogen nitrified, will be the criterion. The data obtained are all given in table 21. More detail concerning the soils will be found in table 24, and a summary of table 21 in per cent of nitrogen nitrified is given in table 22.

TABLE 22

Soil and fertilizer nitrogen nitrified

INCREASE OF NITRIC NITROGEN		SOIL NITROGEN ALONE	DRIED BLOOD ALONE	BONE MEAL ALONE	AMMONIUM SULFATE ALONE	DRIED COWPEA HAY ALONE
p. p. m.	*per cent*	*number of soils*	*number of soils*	*number of soils*	*number of soils*	*number of soils*
Below 0.7	Below 1	15	1	0	3	2
0.7–7	1–10	39*	6	10	4	12
7–14	10–20		1	3	4	6
14–21	20–30		5	5	1	
21–28	30–40		3	6	7	5
28–35	40–50		6	8	5	4
35–42	50–60		5	3	4	
42–49	60–70		5	3	5	1
49–56	70–80		3	1	1	0
56–63	80–90		1	3	2	0
63–70	90–100		3	1	3	0
Loss from all soils............			15	8	14	18
Loss from virgin soils...........			11	5	10	11
Stationary..................			0	1	1	0
Total number of soils tested..		54	54	54	54	54

* The soil nitrogen nitrified was not above 5.2 per cent in any instance. This is much below the results obtained by Lipman and his co-workers.

Discussion of results

The soil nitrogen has not been nearly as well nitrified as that of the added fertilizers. In no case is the amount higher than 5 per cent.

If the summary of the results on these 54 soils is compared with the data of Lipman and his colleagues (25) the South African soils show up badly, as far as their efficiency in converting the soil nitrogen into nitrate is concerned. These American workers found that of the 45 soils from humid areas, 68 per cent transformed 10 per cent and more of the soil's own nitrogen into nitrate, under the same conditions that the writer used. The 150 California soils on which they worked, representing arid and semi-arid parts, came from four soil areas. Forty-seven per cent of the soils of the Riverside area, and 34 per cent of the soils of the Pasadena area transformed 10 per cent and more of the soil's nitrogen into nitrate.

These figures all show the greatly superior nitrifying powers of the soils from humid and semi-arid areas of North America, when compared with South African soils. The soils of the humid area far outclass those of the arid regions of California, which, again, far surpass ours.

Sackett of Colorado (39), in comparing the nitrifying efficiency of 23 Colorado soils with 22 soils from outside that state, as far as they were able to nitrify ammonium sulfate, ammonium carbonate, and ammonium chloride, concludes that the Colorado soils are much superior.

Lipman and his co-workers, however, show that if Sackett's figures are compared with theirs on the basis of soil nitrogen nitrified, only 21 per cent are found that produced 70 parts per million and upwards of nitric nitrogen, whereas 45 per cent of the Pasadena soils did this. When the writer's figures are judged by the same standard, only 3.7 per cent qualify.

Compared with Frap's results on the percentage of soil nitrogen nitrified by Texas soils in 4 weeks, the South African soils are somewhat superior.

Nitrification of the added fertilizers

When table 22 is examined, it will be seen however, that the dried blood, bone meal and ammonium sulfate were well nitrified and gave much better results than the cowpea hay. The cowpea hay was added absolutely dry, a distinct disadvantage, as in the field it is turned under green and decomposes more easily in that condition. Whiting and Schoonover (49) have recently published a paper in which they show that cured clover tops do not decompose and nitrify as easily as green clover tops. When the other three ferilizers are compared with one another as regards the amount of nitrate nitrogen produced over 40 per cent then the dried blood and the ammonium sulfate give equal results and the bone meal is a close third. But when compared on a 50 per cent and upwards basis, then dried blood is first, ammonium sulfate second, and bone meal third.

These results are not in agreement with those of Lipman, Burgess and Klein, who found that dried blood was the worst nitrified of all the substances they used with arid soils. They do agree, however, with Kelly's work, in that small quantities of dried blood, more in accordance with field practice, were well nitrified by semi-arid soils.

The writer's results are compared with Lipman and his colleagues' work in one more respect, viz., in regard to the actual losses of nitrate recorded where fertilizers were added.

TABLE 23

Soils producing less nitrate with fertilizer plus soil nitrogen than from soil nitrogen alone

SOIL	DRIED BLOOD	BONE MEAL	AMMONIUM SULFATE	COWPEA HAY
	per cent	*per cent*	*per cent*	*per cent*
Cultivated and virgin	27.7	14.8	25.9	33.3
Virgin alone	20.3	9.2	18.5	20.3

Dried blood in small quantities has not shown such a high percentage of loss as it did in the four California soils, viz., 35, 43, 63 and 50 per cent. With ammonium sulfate, except in one instance, the percentage loss was higher with our soils. The losses from cowpea hay also were greater in number than those produced by cottonseed meal.

In the South African soils, the losses in most cases were after the addition of the fertilizers to virgin soils. When the nitrifying powers of the virgin

soils alone are considered, the order of merit of the fertilizers is somewhat different, bone meal being first, ammonium sulfate second, and dried blood third; cowpea hay keeps its fourth place. This seems to indicate that on a newly broken-up veld soil, when sufficient time has elapsed to make fertilizing advisable, bone meal may be the best substance to apply if a nitrogen fertilizer is required.

In most instances, the cultivated soil was best nitrified. In several, where the cultivated soil was well nitrified, the virgin soil of the same type was practically at a standstill. Some soils, however, show better nitrification in the virgin than in the cultivated soil of the same type.

In table 24 will be found more detailed data concerning the 54 soils; the locality from which they came, the soil type, general remarks, the rainfall of the area, the loss on ignition, the nitrogen percentage of the soil, the hygroscopic moisture and the Veitch lime requirement.

The writer thought that he might be able to correlate some of these data with the efficiency with which nitrification took place in the soil. However, he has been unable to establish any relationship between the rainfall, the organic matter, the nitrogen content, and the hygroscopic moisture content of the soil, and its nitrifying power. Some of the soils that nitrified worst, came from the areas of highest rainfall, some of the best from the areas of lowest rainfall. Some soils with a low organic matter and nitrogen content gave a high percentage for the nitrification of soil nitrogen; some richer soils gave a very small percentage of nitrification. There seems to be more relationship between the nitrifying power and the lime requirement than between the latter and any of the other properties already mentioned. Even this relationship is not very striking, e.g., the nitrogen in soil 39, with a lime requirement of 14,400 pounds per acre, was well nitrified, and soil 54, with a lime requirement of 1124 pounds per acre, produced more nitrate nitrogen from its soil nitrogen than any other soil. The Winkle Spruit soils, no. 9, 10, 11 and 12, are both acid and alkaline, yet the most efficient nitrification was produced in one of the acid soils. Then again there are some soils that apparently follow the lime requirement as far as nitrification goes, i.e., good nitrification in alkaline soils and poor nitrification in those of a fairly high lime requirement.

A further study, made on the basis of the lime requirements, and in which lime is added, may give more interesting results. It seems, however, that very good nitrification goes on in soils which have quite a high lime requirement, according to the Veitch method.

Summary

1. The nitrification of the soil nitrogen of the 45 humid soils, as recorded by Lipman and his co-workers, was much superior to the results which the writer of this paper obtained with 54 South African soils.

TABLE 24

Some details of soils used in experiment X

NUMBER	LOCALITY AND SOIL TYPE	REMARKS	LOSS ON IGNITION	NITROGEN	HYGROSCOPIC WATER	VEITCH LIME REQUIREMENT (PER ACRE-FOOT)
			per cent	*per cent*	*per cent*	*pounds*
	Rustenburg Experiment Farm.					
1	Coarse yellowish brown sandy loam, virgin	Representative of large areas of the district; rainfall, 25.86 inches	3.40	0.047	0.5	A
2	Light yellowish brown coarse sandy loam, cultivated		3.27	0.075	0.9	580
	Glen Experiment Farm, O. F. S.					
3	Greyish brown clayey loam, virgin, 10 yards from no. 4	At edge of irrigated soil getting about 20 inches rainfall per annum	4.75	0.107	3.5	A
4	Greyish brown clayey loam, cultivated	Irrigated land, cultivated 4 years, crops good	4.73	0.084	2.6	A
	Glen Experiment Farm, O. F. S.					
5	Reddish brown fine sandy soil, virgin	Representative of the Glen dry lands; rainfall 18.3 inches	2.05	0.035	1.0	A
6	Light-reddish brown fine sandy loam, cultivated	Growing trees, Arizona, cultivated annually, dry lands	2.42	0.054	1.4	A
7	Same, virgin	Grass land	14.10	0.225	3.6	8127
	Cedara Experiment Farm, Natal.					
8	Brown silty loam	Cultivated 1 year, rainfall 33 inches	14.53	0.249	3.6	6385

	Winkle Spruit Experiment Farm.					
9	Light brown fine sandy soil, virgin	Typical of Natal south coast soils	1.1	0.023	0.1	174
10	Same, cultivated	Rainfall 45 inches	2.20	0.050	0.6	2322
11	Brown fine sandy soil, virgin	From hillside	1.48	0.049	0.3	A
12	Light brown fine sandy loam	From hillside	1.05	0.033	0.1	A
	Mulder's Vlei.					
13	Coarse sandy greyish brown clay loam, virgin	Typical of wheat soils toward Malmesbury, rainfall 30.52 inches	3.00	0.075	0.7	2300
14	A coarse sandy greyish brown clay loam, cultivated	Cultivated for over 40 years, rainfall 30.52 inches	2.65	0.064	0.7	3900
	Grootfontein Experiment Farm.					
15	Middelburg Cape. Karroo red sandy loam, virgin	Typical of large areas in Karroo, rainfall 10 inches	6.31	0.102	3.4 A	A
16	Same, cultivated		5.64	0.092	2.9	A
17	Same, virgin		2.67	0.105	3.0	A
18	Same, cultivated		5.89	0.109	2.8	A
	Klerksdorp.					
19	Red sandy loam, virgin	Good average fertility and typical of large areas, subsoil grave land clay, rainfall 21.89 inches	4.53	0.087	0.6	2730
20	Dark red sandy soil, cultivated		4.30	0.078	0.7	1400

TABLE 24—*Continued*

NUMBER	LOCALITY AND SOIL TYPE	REMARKS	LOSS ON IGNITION	NITROGEN	HYGROSCOPIC WATER	VEITCH LIME REQUIREMENT (PER ACRE-FOOT)
			per cent	*per cent*	*per cent*	*pounds*
	Rustenburg.					
21	Coarse reddish brown sandy loam, virgin	A little darker than the cultivated soil; near Wolhuters Kop, northern slopes, Magaliesburg; irrigated citrus land, soil 12 feet deep, rainfall 29.16 inches	2.95	0.043	0.3	819
22	Coarse red sandy loam, cultivated		2.55	0.029	0.2	1080
	Kuruman.					
23	Grey fine sandy loam, virgin	Darker than cultivated soil below near Kamden Vryburg; calcareous subsoil 2 feet, irrigated land, for 8 years grew general crops, mangels and Sudan grass grow well, rainfall 19.69 inches	5.60	0.096	1.0	Contains 6.48 per cent $CaCO_3$
24	Light grey fine sandy loam, cultivated		5.54	0.136	1.2	9.3 per cent $CaCO_3$
	Standerton.					
25	Dark brown clay loam, virgin	From near Holmdene; a typical high veld soil, rich chemically but not easy to work, chief crops maize and teff, at 12 to 18 inches, the subsoil becomes darker and more clayey, rainfall 24.33 inches	5.86	0.204	5.4	1620
26	Same, cultivated		5.25	0.154	4.9	2160

	Krugersdorp.					
27	Brown medium fine sandy loam, virgin	Near Magaliesburg station; cultivated and irrigated citrus, rainfall 27.78 inches	3.57	0.059	0.1	1530
28	Reddish brown medium fine sandy loam, cultivated		3.01	0.054	0.2	700
	Pietersburg.					
29	Coarse light brown sandy loam, virgin	Rainfall 21.69 inches, cultivated 5 years, maize, wheat and beans	1.96	0.052	1.3	A
30	Same, cultivated		2.09	0.048	1.2	A
	Ventersdorp.					
31	Dark chocolate loam, virgin	This soil type occurs in circular depressions of about 100 yards diameter, overlying dolomite; manganese pebbles in subsoil, occurs north of Black Reef, rainfall 23.46 inches.	4.95	0.083	0.6	A
32	Same, cultivated		3.65	0.064	0.6	1220
	Koekemoer.					
33	Red medium fine sandy loam, virgin	Virgin soil slightly darker, rainfall 23.59 inches, food fertility with good rain, easily blown and infested with ants	3.17	0.061	0.6	740
34	Same, cultivated		2.61	0.048	0.3	546
	Lichtenburg.					
35	Chocolate fine sandy loam, virgin	Good fertile soil representative of best in district, chief crop maize; underlain by dolomite, rainfall 23.74 inches	2.89	0.084	1.4	543
36	Same, cultivated		2.71	0.072	1.4	1086

TABLE 24—*Concluded*

NUMBER	LOCALITY AND SOIL TYPE	REMARKS	LOSS ON IGNITION	NITROGEN	HYGROSCOPIC WATER	VEITCH LIME REQUIREMENT (PER ACRE-FOOT)
			per cent	*per cent*	*per cent*	*pounds*
	Ladybrand.					
37	Light brown fine sandy loam, virgin	Derived from Cave sandstone; fine soil physically and also good chemically, rainfall 27.34 inches	2.26	0.057	0.5	273
38	Same, cultivated		2.36	0.069	0.7	546
	Near Johannesburg.					
39	Kliptown black peaty loam, virgin	A very rich soil but has a large lime requirement, rainfall 28.4 inches	21.13	0.764	5.7	14400
	Rosetta, Natal.					
40	Light brown silt loam, virgin	Darker than cultivated, 8 inches deep, sour veld, rainfall 31.33 inches, yields good crop of potatoes with superphosphate and stable manure	18.06	0.359	3.8	8625
41	Same, cultivated for 5 years		11.83	0.303	6.3	8992
	Marquard O. F. S.					
42	Light brown fine sandy loam, virgin	Of Cave sandstone origin; depth around 3 feet, then limonite and clay	2.45	0.061	0.2	287
43	Same, cultivated	Cultivated 35 years, darker in color than virgin, rainfall 22.95 inches	2.35	0.058	0.1	575
	Blythswood Transkei.					
44	Grey sandy loam, virgin	Soil about 2 feet, gravel and stony subsoil	3.0	0.092	0.9	287
45	Light grey sandy loam, cultivated	Cultivated 20 years, not renowned for fertility, rainfall 25.43 inches	2.12	0.064	0.1	1150

	Meimoth, Zululand.					
46	Brown loam, virgin	Virgin soil darker than cultivated, 2 feet deep red subsoil; crop yields poor; rainfall 29.61 inches	11.75	0.162	2.4	3162
47	Same, cultivated		10.39	0.160	2.2	4600
	Escourt, Mooi River.					
48	Light brown clay loam, virgin	Virgin soil darker than cultivated; both soils have a large amount of small round limonite pebbles; root crops and teff; cultivated about 6 years, soil 2 to 6 feet deep, rainfall 28.97 inches	5.34	0.116	1.3	1725
49	Same, cultivated		3.34	0.094	1.5	1100
	Molteno.					
50	Light brown sandy loam, virgin	Soil 18 to 24 inches deep, underlain by subsoil of 10 feet, then sandstone; wheat and barley grown, rainfall 21.22 inches	2.77	0.085	1.0	278
51	Dark brown clay loam, cultivated		3.66	0 138	4.1	A
	Potchefstroom.					
52	Dark greyish black clay loam, virgin	Very fertile soil, typical of irrigated soil in distribution	9.93	0.200	4.0	Contains 18.9 per cent $CaCO_3$
53	Black clay loam, cultivated		5.14	0.157	3.2	5.93 per cent $CaCO_3$
	Potchefstroom.					
54	Brown sandy loam, cultivated	Fallow strip of dry land plots studied for seasonal variation	6.50	0.100	1.9	1124

2. Even the results from the majority of the soils of the semi-arid parts of California and Colorado are superior to the writer's figures, which are, again, better than the data from Texas soils.

3. It does not seem, from this study that nitrification in South African soils is as intense as has been hitherto supposed.

4. Dried blood, in small quantities, was the most efficiently nitrified of the four fertilizers used. Ammonium sulfate, bone meal and dried cowpea hay followed in order of merit.

5. With the virgin soils, bone meal was best nitrified, then ammonium sulfate, dried blood, and dried cowpea hay.

6. Nitrification in the cultivated soils was, in general, superior to that in the virgin soils.

7. No relationship could be established between the efficiency with which the soil nitrogen was transformed to nitrate, and the organic matter, the nitrogen, and hygroscopic moisture content of the soil, or the rainfall of the area from which the soil came.

8. There seems a perceptible relationship, in some cases at least, between the amount of nitrate produced from soil nitrogen, and the Veitch lime requirement of the soil.

FINAL DISCUSSION AND CONCLUSIONS

The seasonal variation data seem to point to better and more active nitrification at Potchefstroom than at Rothamsted. In this the writer agrees with Watt but he cannot endorse that worker's opinion that nitrification in Transvaal soils is in general superior to that in the soils of most temperate climates. One reason is that seasonal-variation field data from Ithaca, N. Y., record very much superior amounts of nitric nitrogen than were found here.

Again, when 54 South African soils were incubated, most of them under the same conditions as used by Lipman and his co-workers, the resulting data were much inferior to those obtained by these investigators on 45 soils from humid areas of the United States. They also found, on using the actual percentage of soil nitrogen nitrified as the criterion, that these 45 soils from humid areas were superior to soils from the arid and semi-arid sections of California.

The writer had always believed that nitrification in South African soils was very active and had hoped to find it so. From the field and laboratory data obtained in these studies, however, the following conclusions seem reasonable:

Conclusions

1. Nitrification in South African soils, when compared with that in soils from many other parts, cannot be said to be exceptionally active, although it is good compared with data from some areas.

2. These data add further evidence from another section of the globe, to that recently accumulated by workers in Western America, to show that nitrification is not so intense in the soils of arid and semi-arid regions, or always superior to that in the soils of humid areas.

3. However, nitrification seems quite adequate for the average crop production in this area of summer rainfall. The last study showed that 5 per cent of the soil's nitrogen on this farm could be nitrified under optimum conditions in 30 days. That means a capacity for producing about 180 pounds of nitric nitrogen per acre foot. This, though, is a higher capacity than was shown by most samples of the soils tested; nitric nitrogen was always found on the dry-land plots even at the time of the most active growth of the maize crop.

4. Where the maximum rainfall and temperature coincide, there is maximum nitrification at the time of greatest crop growth. Thus it seems that there is not much need of nitrogenous fertilizers in the summer rainfall area. On the other hand, farm practice shows great benefit from nitrogenous manures in the Cape areas of winter rainfall, where the optimum conditions of moisture and temperature occur at different times.

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INOCULATED LEGUMES AS NITROGENOUS FERTILIZERS

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The beneficial effects of the growth of legumes on the yields of subsequent crops has been known for centuries. Many explanations were suggested from time to time to account for this peculiar influence of leguminous crops in comparison with non-legumes, but none proved very tenable. It was not until the eighties of the last century that an adequate explanation was offered. The discovery, at that time, of the ability of certain bacteria when growing in nodules on the roots of legumes, to fix the free nitrogen of the atmosphere and furnish it to the plant, served to show that legumes benefit the soil mainly because, when inoculated, they increase the nitrogen supply available for succeeding crops.

This discovery also provided the explanation for many peculiar occurrences which had been noted in the growth of legumes. The ability of such crops to grow without nitrogen was found to depend upon the presence of the proper bacteria and the reason for the failure of crops in some cases and the successful growth in others under apparently similar conditions of low nitrogen supply, became evident. The large benefits occurring in some cases from the use of legumes and the absence of effect in other instances also was explained. The cause of the beneficial effect on clover of spreading soil from an old cultivated field over newly broken land was found to be due to the bacteria introduced. In short, the "abnormal behavior" of legumes, noted so many times in investigational work and in practice, was easily explainable when viewed from a bacterial standpoint.

Following this discovery of the symbiotic relation between bacteria and legumes, a large amount of investigational work began and has continued to the present time. The original experiment itself has been checked by numerous investigations and the accuracy of the conclusions drawn has been satisfactorily proven. The subject of soil inoculation has received much attention, and has led to the very practical conclusion that if legumes are to be successfully grown and well inoculated the proper bacteria must be supplied by inoculation in all cases where they are not present.

Cross-inoculation, the grouping of the bacteria from different legumes into classes, the preparation of highly efficient or virulent cultures of organisms, the non-symbiotic nitrogen-fixing power of the bacteria, the relation of the symbiotic process to the nitrogen content of the soil, the length of time during which the

organisms remain alive and vigorous in the soil and the isolation of the bacteria from the soil, are some of the more or less technical phases of the subject which have received attention. From the practical standpoint the problem of chief interest has been the relation of the growth of inoculated legumes to soil fertility and particularly to permanent agriculture. Secondary to this has been the problem of the proper soil conditions for the best growth and inoculation of legumes.

The need for a return of nitrogen to the soil in an amount equal to that removed by crops is a recognized fact in all systems of permanent fertility. This return may be accomplished by the use of artificial or natural nitrogen carriers. Commercial nitrogenous fertilizers are expensive and if the nitrogen of the atmosphere can be utilized by the growing of inoculated legumes, the nitrogen supply in the soil can be kept up much more economically. Furthermore, the legumes not only supply nitrogen but also add organic matter to the soil and thus have a double value. Practically all systems of farming at the present time therefore, include the use of legumes in the rotation and it is quite generally accepted that *by proper handling of these crops* either as a definite part of the rotation or as green manures, the nitrogen supply in the soil may be very largely maintained without the use or by only small additions of commercial nitrogenous fertilizers. It is admitted that such materials may prove profitable in some cases for special soil and crop conditions but on the *average* soil for general farm crops they are considered as supplements to inoculated legumes.

While it is commonly believed, therefore, that inoculated legumes add nitrogen to the soil and that these crops are very valuable nitrogenous fertilizers, it is a matter of considerable interest to note that there is very little definite knowledge regarding the actual amount of nitrogen which legumes add to the soil. There has been much theorizing, and many assumptions have been made but without any very definite background of fact for field conditions. A pamphlet published several years ago (7) calls attention very strikingly to the dearth of accurate information on this important point.

It is obvious that if we are to depend upon legumes to supply most of the nitrogen needed for the permanent fertility of soils, considerable data must be secured by extensive experiments which will show the actual amount of nitrogen which legumes take from the atmosphere or rather the relative amounts secured from the atmosphere and from the soil under a wide range of soil conditions, particularly as regards nitrogen content. It is probably true, as is now believed, that on soils rich in nitrogen less of that element is taken from the air than is the case on soils low in nitrogen. It is probably true also that it is more difficult to secure inoculation of legumes on rich soils than on soils low in nitrogen and organic matter. Some information has been secured regarding the relative nitrogen content of the tops and roots of legumes, but further data along this line should be secured under control conditions with different legumes and on various soils, if definite recommendations,

regarding the proper handling of legumes to secure the best effects on soils, are to be made. Again, practice in this matter is based upon assumptions rather than upon the results of scientific experiments.

The nitrogen problem in permanent agriculture cannot be solved until there is much further experimental work carried out along the lines indicated. Then and only then will it be possible to make definite and complete recommendations which will stand the test of long-continued practice.

The work reported in the following pages was planned to secure preliminary information on some of the problems mentioned above, and while the results are of course far from complete, they are presented here inasmuch as they indicate quite clearly the nitrogen fixed by two common inoculated legumes on two extensive soil types in this state, and as they show also the distribution of nitrogen between the tops and roots of these legumes, at various stages of growth, under control conditions.

HISTORICAL

It is not necessary to give an extended bibliography of the investigations dealing with the symbiotic fixation of nitrogen or rhizofication, as very complete lists of publications have been given in reports on various phases of the subject. It seems desirable, however, to review briefly the results of previous experiments which throw any light on the particular problems studied in this work. The references given are only those which contain information on the amount of nitrogen fixed by inoculated legumes as determined by the relative nitrogen content of inoculated and uninoculated plants, and on the relative nitrogen content of the tops and roots of legumes.

Hopkins (22) suggests the comparison of the nitrogen content of inoculated and uninoculated plants as a method of calculating the nitrogen taken from the air, and assumes that it gives correct results. He also suggests that a comparison of the total nitrogen content of a non-leguminous crop with that of a crop of infected legume plants grown at the same time on the same soil will give practically accurate information on the subject. The latter method can hardly be considered satisfactory for obvious reasons and there are objections to the former, but at least, it may serve as an indication of what may be expected from more exact tests.

Experiments at Rothamsted (42) have given evidence of increased nitrogen in soils from the growth of inoculated legumes. For example, soil from a field where clover was grown showed 0.156 per cent of nitrogen while where barley was grown the content was 0.142 per cent to a depth of 9 inches. This would amount to 350 pounds per acre of 2,500,000 pounds of soil.

Aeby (1) grew peas in a poor soil and in a rich soil and found a fixation of 1.976 gm. of nitrogen per 4 kgm. of soil in the rich soil and 2.759 gm. in the poor soil.

Duggar (11) experimenting with crimson clover and hairy vetch found a fixation of 98.5 pounds of nitrogen per acre in the one case and 139.4 pounds

per acre in the other, calculating the nitrogen in the entire crop including the roots and stubble. With both these crops the yields when inoculation was not practiced were very small.

Experiments on alfalfa in Illinois reported by Hopkins (20) give results in the field tests which show a fixation of about 40 pounds of nitrogen per acre from the air by bacteria—in one crop, on the untreated soil. When lime was added a further slight increase was obtained and with lime and phosphorus, a gain of over 53 pounds was secured. The total fixed for the season was calculated at 172 pounds on the unfertilized plots and 252 pounds on the plots receiving lime and phosphorus.

In pot tests with alfalfa reported in the same bulletin, the nitrogen fixed by bacteria in ordinary soil ranged from 7 to 90 pounds per acre. The average fixation in the unfertilized pots amounted to about 40 pounds. This amount was increased in some cases on the fertilized soils but where nitrogen was supplied the fixation was reduced. The largest amount was fixed when lime, phosphorus, and potassium were applied.

Later experiments of Hopkins (21) with cowpeas seeded as a catch crop after oats on land heavily cropped to corn and oats until nitrogen was the limiting factor of growth, showed that as an average of the results of the analyses of ten immature plants on each of six plots, three inoculated and three uninoculated, 73 per cent of the nitrogen in the inoculated plants came from the air. They showed also a fixation of 12.9 cgm. of nitrogen per plant.

Inoculation experiments with alfalfa at New Jersey (8) showed a 40 per cent gain in two cuttings by inoculating with a soil infusion. A smaller increase was given when soil was used as the inoculum. The crop was not analyzed but assuming the same per cent of nitrogen in both inoculated and uninoculated plants, a large nitrogen fixation was apparent.

Experiments by Shutt (34) in Canada showed a gain in nitrogen in pot tests of 0.0065 per cent, or 130 pounds per acre of 2,000,000 pounds of surface soil, from the growing and turning under of mammoth clover for two successive seasons. In plot tests for two seasons, with two cuttings removed, and all residues returned, there was an increase of 0.0143 per cent, or 286 pounds per acre, an annual fixation of 143 pounds. In later work by the same author (35) the growing of clover on a sandy soil for 6 years was found to increase the nitrogen content of the soil to the amount of 375 pounds per acre.

Nobbe and Richter (28) in one experiment found that 93 per cent of the nitrogen in vetch was fixed from the atmosphere. In a later test, 96 per cent was found to be fixed. Only the nitrogen in the tops of the plants was determined in this work. A gradual increase in the nitrogen fixed occurred up to the maturity of the plants. Tests with additions of varying amounts of nitrogen added showed a decrease in the nitrogen fixed, the decrease becoming greater with the larger additions of nitrates.

Smith and Robison (36) found 33 per cent of the nitrogen in soybeans fixed by bacteria, and with cowpeas 15 per cent of the nitrogen was secured from the air, In one case the nitrogen fixed amounted to 37 pounds per acre and in the other case to 21 pounds per acre. In two other tests with areas 12 feet square, the soybeans (tops only) took 44 per cent and 32 per cent of their nitrogen from the air, showing a fixation amounting to 283 pounds and 134 pounds of nitrogen per acre, respectively.

Studies with soybeans by Woll and Olson (45) at Wisconsin, showed that when grown on a rich soil, 14 per cent of the nitrogen in well inoculated plants came from the atmosphere. The amount of nitrogen fixed amounted to 16 pounds per acre.

In field tests of various commercial cultures for the inoculation of legumes, Lipman (23) found a fixation of 13 pounds of nitrogen per acre in the case of cowpeas. With alfalfa, a fixation of 15 pounds of nitrogen was found when soil was used for inoculating and 35 pounds when a commercial culture was employed, lime being applied to the soil in both cases. In tests with cowpeas, soybeans and bush limas in rows, there were considerable increases in the nitrogen fixed as indicated by the total nitrogen in the grain.

Alway and Pinckney (4) found a fixation of 92 per cent of the nitrogen in alfalfa plants through inoculation. The fixation amounted to 0.004107 gm. per plant.

Alway and Bishop (3) report no marked difference between the amounts of nitrogen in soils from an alfalfa field and from a corn field. Later work at Kansas by Swanson (38) and by Swanson and Latshaw (39) shows that the growing of alfalfa for 20 or 30 years did not add to the nitrogen in the soil but neither did it reduce it to any extent. Where grain had been grown continuously the soil was 21.6 per cent lower in nitrogen than under alfalfa but in the latter case there was 14.3 per cent less nitrogen than in the native sod.

Hartwell and Pember (19) determined the gain in nitrogen in a pot experiment with different legumes over a 5-year period. Cowpeas and soybeans were grown each summer and vetch was grown in the pots each winter and turned under in the soil. The approximate net gain in the presence of these crops was 1 ton of nitrogen per acre, seven-tenths of which was contained in the 25 tons of moisture-free summer crops removed and the remainder in the soil.

Lyon and Bizzel (27) grew alfalfa and timothy for 6 years and then planted corn and oats. The corn yielded 15 bushels more on the alfalfa soil while the oats showed practically no difference. The alfalfa soil contained 0.01 per cent more nitrogen than that under timothy and this would amount to a fixation of 200 pounds per acre of 2,000,000 pounds of surface soil.

Arny and Thatcher (5) studying different methods of inoculating alfalfa, found in two field tests on the Minnesota University Farm, a fixation of 18 pounds of nitrogen per acre, as an average of all methods. In another test

a fixation of 48 pounds of nitrogen per acre was noted. With sweet clover there was a fixation of 23 pounds per acre. In a second year's work on the same soils (6) 10 pounds of nitrogen per acre was fixed by alfalfa compared with 18 the first year. In the second test with the same crop 37 pounds was fixed against 48 the preceding year. Calculations based on analyses of the entire plants of alfalfa and sweet clover, from various areas of 1 square yard each, show 118 pounds of nitrogen fixed per acre by alfalfa while with sweet clover 76 pounds was fixed. When lined, the sweet clover showed a fixation of 133 pounds of nitrogen per acre.

Lipman and Blair (24) using cylinder experiments with various legumes turned under as green manures in a rotation of corn, potatoes, oats and rye, found a gain of 54 pounds of nitrogen annually over a period of 7 years.

Later experiments by the same authors (25) with soybeans grown in pots on a poor sandy soil and on a good silt loam, showed increases up to 624 mgm. and 208 mgm. of nitrogen, respectively, with various commercial cultures and with soil used for inoculation. On the poor soil the average fixation with all inoculants was 333 mgm., or 139 pounds per acre, while with the richer soil only 78 mgm., or 32 pounds per acre, was fixed on the average.

Pot tests by Fred and Graul (16) at Wisconsin, showed a gain of 20 pounds of nitrogen per acre from inoculated alfalfa and 59 pounds per acre when lime was applied. With soybeans in similar pot tests there was a fixation of 33 pounds of nitrogen per acre resulting from inoculation and of 64 pounds per acre when lime was applied. Later experiments with alfalfa on an acid Colby silt loam showed a fixation of 140 pounds of nitrogen per acre from inoculation and 245 pounds per acre when lime was applied. When the nitrogen of the roots was determined also the fixation became 164 pounds and 264 pounds respectively. On Sparta sand, the increases from inoculation were 81 pounds and 269 pounds per acre with and without lime for the tops alone, and for tops and roots 105 and 339 pounds per acre. With red clover on the Colby silt loam the fixation by inoculation was 5.9 pounds and 62 pounds per acre with and without lime, while on the Sparta sand the figures were 54 pounds and 145 pounds of nitrogen fixed. The nitrogen present in the soils used in these various pot tests was determined at the beginning and at the end of the experiment, and from a calculation of the nitrogen in the seeds and in the crops removed, the actual gain in nitrogen in the experiment was calculated. With alfalfa the amounts fixed from inoculation were 206 pounds and 70 pounds per acre without lime, and 180 pounds and 323 pounds with lime. With clover the amounts were 5 pounds and 160 pounds per acre without lime, and a loss of 15 pounds and a fixation of 214 pounds with lime.

Blair (9) studied the effect of both cowpeas and soybeans as green manures on the yields of wheat and rye in pot tests and found an average gain of 15 pounds of nitrogen annually for the two crops, for the last 4 years of an 8-year period. The 8-year average amounted to about 12 pounds.

Brown (10) found that the inoculation of alfalfa by various cultures and by soil brought about an average fixation of 17 pounds of nitrogen per acre and 14 pounds per acre in two series of plot tests. With cowpeas, the fixation amounted to 28 pounds and with soybeans to 41 pounds of nitrogen per acre.

Experiments in pots by Fellers (15) showed an increase of 1 to 3 per cent in the protein content of the seed of soybeans with various inoculants. In field tests, increases up to 9.5 per cent of protein in the seeds were noted.

Fred and Graul (18) in pot tests with soybeans on Hancock sand found with one crop an average increase of 100 pounds of nitrogen per acre from inoculation. Determinations of the source of nitrogen in the soybeans, by analysis of soil, seed and crop showed, for the three crops grown, that 323 pounds of nitrogen per acre was taken from the atmosphere by inoculated plants while when lime was used, 389 pounds per acre was secured. In a field test an average increase of 24 pounds of nitrogen per acre was found from inoculation.

Albrecht (2) found in the soil tested after growing one crop of soybeans and two of cowpeas, an increase in nitrogen of 107 pounds per acre. Where clover tops were added to the soil the fixation was smaller. After the first crop was grown there was a small decrease in nitrogen, but following the second crop a considerable increase occurred. It should be noted that the decrease was much smaller than the error in the determination of nitrogen while the increases were much beyond it.

It is apparent from the experiments cited that the amount of nitrogen fixed by legumes, as measured by the increased nitrogen in inoculated over uninoculated crops, is extremely variable and depends upon many soil and crop conditions. Little information is supplied, however, which shows definitely the proportion of nitrogen in inoculated legumes which is taken from the air and the proportion from the soil. Only in those tests where the nitrogen content of the soil is determined before and after the growth of the legumes is there really definite data on this point. Again the composition of the soil and other factors undoubtedly influence the results secured.

Considerable work has been carried out in studying the relative nitrogen content of the tops and roots of legumes, and also the relation between the weights of the tops and the roots. A brief summary of previous investigations along this line will be given here.

Lupton (26) in experiments with peas found, as an average of four tests, 8 per cent of the nitrogen in the roots and stubble and 92 per cent in the tops. Tests in New Jersey reported by Voorhees and Street (40) show 6 per cent of the nitrogen of cowpeas in the roots and 94 per cent in the vines. Experiments with crimson clover the succeeding year (41) show 28 per cent of the nitrogen in the roots on April 24, 8 per cent on May 12, 6 per cent on May 24 and 5 per cent on May 31.

Snyder (37) found 20 per cent of the nitrogen in clover in the roots. Waters (43) reported 32 per cent of the nitrogen in clover hay in the roots and stubble

while with crimson clover 20 per cent was in the roots and stubble. Roberts and Clinton (33) found 19 per cent of the nitrogen of crimson clover in the roots, 39 per cent with red clover and 54 per cent with mammoth clover. Duggar (11) found 16 per cent of the nitrogen of crimson clover in the roots and stubble while with hairy vetch, 18 per cent was in the roots. In a later experiment (12) with velvet beans, 6 per cent of the nitrogen was in the roots and stubble and 94 per cent in hay. In a still later test by the same investigator (13) with hairy vetch, 14 per cent of the nitrogen was in the roots and stubble on April 19, just before blooming, 13 per cent on April 26 when 5 per cent of the blooms were showing, 11 per cent on May 2 at full bloom, and 14 per cent on May 14 when seed pods were formed but not filled.

Woods (46) determined the nitrogen in the tops and roots of various legumes and found the proportion of nitrogen in the roots of horse beans to be 16 per cent, soybeans 5 per cent and 18 per cent, cowpeas 10 per cent, vetch 15 per cent, white lupines 10 per cent, yellow lupines 10 per cent, blue lupines 9 per cent and red clover 24 per cent.

Duggar (14) testing the use of cowpeas as a fertilizer found that at the blooming stage 7 per cent of the nitrogen was in the roots and stubble, 78 per cent in the vines and 15 per cent in the fallen leaves and leaf stalks. At the ripening stage the corresponding figures were 9 per cent, 65 per cent and 26 per cent. Penny (29) grew crimson clover on a sandy soil and on a clay soil, and on April 22, he found 31 per cent of the nitrogen in the roots on the sandy soil and 22 per cent on the clay soil. On May 22 the corresponding figures were 29 per cent and 11 per cent. He suggests that the difference in the case of the two soils is partly due to the greater ease of recovering the roots on the sandy soil and to the fact that the tops of the plants increased less on the sandy soil in the interval of sampling. In later tests with various legumes as cover crops the same investigator (30) found with soybeans 6 per cent of the nitrogen in the roots, with cowpeas 6 per cent, with vetches, 10 per cent, with crimson clover 5 per cent, with alfalfa 42 per cent, and with red clover 32 per cent.

Hopkins (21) in experiments with cowpeas found that average results from three different lots of 10 plants each, showed that 86 per cent of the nitrogen in inoculated plants was in the tops, 5 per cent in the roots and 9 per cent in the tubercles, while with the uninoculated plant, 7 per cent was in the roots and 93 per cent in the tops. In tests with red clover the same author (22) reports 25 per cent of the total plant nitrogen in the surface roots (0-7 in.) while only 1 per cent was in the lower roots. With cowpeas, 12 per cent was in the subsurface roots and one per cent below and with soybeans, the corresponding amounts were 8 per cent and 1 per cent. With sweet clover 14 per cent of the total nitrogen was found in the roots and 86 per cent in the tops.

Experiments by Shutt (34) showed 19 per cent of the nitrogen in poorly inoculated horse beans in the roots, and 25 per cent in better inoculated plants.

With mammoth red clover, 40 per cent of the nitrogen was in roots. In a field test, mammoth red clover seeded with barley, showed the subsequent year 28 per cent of the nitrogen in the roots.

The most complete study of any legume was made of crimson clover by Penny (31). He found that the proportion of nitrogen content of the roots to that of the whole plant ranged from 12 to 50 per cent and averaged about 30 per cent. The proportion fluctuated greatly and had little connection with the stage of growth. The yield of nitrogen 30 days before full bloom when the crop was on the average just half grown, ranged from one-half to four-fifteenths of the yield at full bloom. On soil accustomed to the crop little nitrogen was gained during the last month, while on new soil much was taken up late in growth. In one experiment the proportion of nitrogen in the roots 32 days before full bloom was 19 per cent, 27 days beore full bloom 20 per cent, 18 days before full bloom 1.9 per cent, 8 days before full bloom 25 per cent, at full bloom 26 per cent and 30 days after full bloom, 20 per cent.

In a second test 29 days before full bloom, 34 per cent was in the roots, 20 days before full bloom 34 per cent, 8 days before full bloom 36 per cent and at full bloom, 43 per cent. In a third test 24 days before full bloom, 22 per cent was in the roots, and at full bloom, 25 per cent. In a fourth test the figures for 25 days before and at full bloom were 32 per cent and 39 per cent, respectively. In a fifth test, 26 days before full bloom, 22 per cent was in the roots while at full bloom only 11 per cent was found. In every case the total nitrogen present in the entire plant increased up to full bloom and then decreased. On the average 25 per cent of the total nitrogen of the crop was underground. When the stubble and roots were plowed under it was assumed that 35 to 40 per cent of the nitrogen was added to the soil. No information is given as to the amount of "new" nitrogen taken up by the plant, although it is suggested that the amount of "new" nitrogen is probably smaller on a soil rich in that element.

Smith and Robison (36) in three tests found 4 per cent, 6 per cent, and 6 per cent of the nitrogen in inoculated soybeans in the roots, while with inoculated cowpeas 9 per cent of the nitrogen was in the roots. Woll and Olson (45) found 4 per cent of the nitrogen in inoculated soybeans in the roots, while in uninoculated plants 1 per cent was in the roots. Penny and MacDonald (32) in experiments with crimson clover found 19 per cent of the nitrogen in the roots in the first fall growth. The next year at the middle stage of growth there was 31 per cent in the roots and at full bloom, 25 per cent. Alway and Pinckney (4) found 31 per cent of the nitrogen of alfalfa plants in the roots on August 3, the first year, while on June 4 of the next year 17 per cent was in the roots. Wiancko, Fisher and Cromer (44) found 11 per cent of the nitrogen in soybeans and 14 per cent of that in cowpeas, in the roots.

Arny and Thatcher (5) experimenting with alfalfa showed 25 per cent of the nitrogen on the average in the roots, with sweet clover 12 per cent was in

the roots. In later experiments by the same investigators (6) with inoculated alfalfa, 21 per cent of the nitrogen was in the roots, but with sweet clover they found only 1.5 per cent in the roots.

Fred and Graul (16) found 31 per cent of the nitrogen in inoculated alfalfa in the roots, while when lime was applied to the soil, 30 per cent was in the roots. On another soil 15 per cent was in the roots when unlimed, while with lime added 10 per cent was found.

Albrecht (2) found with the first crop of cowpeas on an untreated soil, 27 per cent of the nitrogen in the roots. When nitrates were added to the soil the percentage was slightly increased and with larger amounts slightly reduced. In the second crop on the same soil, 15 per cent of the nitrogen was in the roots. Little effect was noted from the addition of nitrates, the smallest application giving a slight increase in the per cent of nitrogen in the roots. On the second soil which had grown soybeans, 21 per cent of the nitrogen was in the roots in the first crop and 17 per cent in the second. With additions of clover tops to the soil, the per cent in the roots was reduced gradually in the first crop, the largest amount showing the greatest reduction. With the second crop a reduction occurred but it was not consistent and the largest application had the smallest effect.

It is apparent from the data given that the actual proportions of nitrogen in the tops and roots of legumes is extremely variable and depends upon many conditions. Hopkins (22) concludes that on the average one-third of the nitrogen of red clover is in the roots. With alfalfa he believes that a larger amount is in the roots, possibly one-half as much is in the roots as is removed by the crop even when the plants are several years old. With cowpeas and soybeans he suggests that as a rule not more than one-tenth of the nitrogen is in the roots and stubble. These estimates may be fairly accurate but at best they are only estimates and variations of considerable moment may frequently occur. Accurate information along this line can be secured only by many experiments on a wide variety of soils under a broad range of crop conditions. The work reported in the following pages was planned to throw some light on this interesting and important problem by securing analytical data both on legumes and on the soils on which they were grown.

EXPERIMENTAL

Two soils were used in this experiment, both glacial in origin, occurring in the Wisconsin drift soil area. One is classified as the Carrington loam by the Bureau of Soils and the other as Miami fine sandy loam. These soils were chosen because of their different characteristics particularly as regards organic matter content. The former is dark brown to black in color and well supplied with humus and nitrogen while the latter is light in color and much lower in organic matter and nitrogen. Analyses showed 0.2701 per cent of nitrogen in the Carrington loam and 0.1353 per cent in the Miami fine sandy loam.

In the various experiments, clover and alfalfa were grown on these two soils, in some cases without treatment, that is, unsterilized and uninoculated, and in the others sterilized and inoculated with cultures containing the proper bacteria to bring about inoculation. The sterilization was accomplished by autoclaving for 1 hour.

Large samples of the two soils were secured from the field, sieved and either filled into pots directly or sterilized and then weighed in pots. Eight pots were filled with about 10 pounds of soil in each test, enough soil being employed so that the pots were well filled, and the exact weight of dry soil was determined in each case. Clover or alfalfa was seeded in the various groups of pots, pure cultures added to the sterilized series and the moisture content was brought up to the optimum by adding water to weight. The moisture content was kept up during the continuance of the experiment by weighing the pots and adding water to weight. The same number of plants were grown in each pot both with the clover and with the alfalfa.

In each test the crop was harvested from duplicate pots at various stages of growth, the times chosen being two weeks before blooming, when the blooms appeared, at full bloom and when mature. The green and dry weights of the crops were secured and the roots were carefully and completely removed from the soil, dried and weighed. The nitrogen content of both the tops and the roots was then determined by the Kjeldahl method on duplicate samples of each. The nitrogen content of the soil also was determined after the removal of the crops. Four determinations were made on each soil and the results were required to agree very accurately, repeats being run in all necessary cases so that the results agreed to the fourth decimal place. Only the final average results are given in the tables.

Series I

In this series clover was grown on the Carrington loam, unsterilized and uninoculated. The weights of crop secured, tops, roots and plants, the per cent of nitrogen in the tops and roots and the total nitrogen in tops, roots and plants are given for each pot in table 1. The averages of these results and calculations showing the per cent of plant growth in tops and roots and the per cent of total nitrogen in the tops and roots, in the duplicate tests are shown in table 2.

Examining this latter table it is evident first of all that the largest increase in crop occurred between 2 weeks before blooming and when the blooms appeared. Further increases occurred up to maturity. About 31 per cent of the total weight of the plants was found in the roots as an average of the results at the four stages of growth. The largest amount, 34 per cent, was in the roots when the blooms appeared. This was followed at the time of full bloom by a decrease to 29 per cent and at maturity 32 per cent was found in the roots. These differences, however, are not large enough to be very definite.

TABLE 1

Clover in unsterilized, uninoculated Carrington loam

POT NUMBER	WEIGHT OF						NITROGEN IN				TOTAL NITROGEN IN					
	Tops	Average	Roots	Average	Plants	Average	Tops	Average	Roots	Average	Tops	Average	Roots	Average	Plants	Average
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*per cent*	*per cent*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	5.19		2.30		7.49		3.190		2.400		0.1656		0.0552		0.2208	
2	5.05	5.12	2.20	2.25	7.25	7.37	3.510	3.350	2.500	2.450	0.1773	0.1714	0.0550	0.0551	0.2323	02265
3	39.30		22.35		61.65		2.780		1.725		1.0925		0.3855		1.4780	
4	48.65	43.97	23.85	23.10	72.50	67.07	2.575	2.677	1.655	1.690	1.2527	1.1726	0.3947	0.3901	1.6474	1.5627
5	70.15		29.81		99.96		2.465		1.593		1.7292		0.4749		2.2041	
6	47.10	58.62	18.85	24.33	65.95	82.95	2.289	2.377	1.379	1.486	1.0781	1.4036	0.2599	0.3674	1.3380	1.7710
7	62.30		30.65		92.95		2.031		1.121		1.2653		0.3436		1.6089	
8		62.30		30.65		92.95		2.031		1.121		1.2653		0.3436		1.6089

TABLE 2

Clover in unsterilized, uninoculated Carrington loam

SAMPLING	AVERAGE WEIGHT			PLANT GROWTH IN		AVERAGE TOTAL NITROGEN IN			TOTAL NITROGEN IN	
	Tops	Roots	Plants	Tops	Roots	Tops	Roots	Plants	Tops	Roots
	gm.	*gm.*	*gm.*	*per cent*	*per cent*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*
Two weeks before blooming	5.12	2.25	7.37	69.47	30.53	0.1714	0.0551	0.2265	75.67	24.33
When blooms appeared	43.97	23.10	67.07	65.56	34.44	1.1726	0.3901	1.5627	75.03	24.97
Full bloom	58.62	24.33	82.95	70.66	29.24	1.4036	0.3674	1.7710	79.25	20.75
Mature	62.30	30.65	92.95	67.02	32.98	1.2653	0.3436	1.6089	78.64	21.36

The total nitrogen in the tops was the greatest at full bloom and decreased somewhat at maturity. In the roots there was the largest amount of nitrogen when the blooms appeared, small decreases occurring up to maturity. In table 1, the percentage of nitrogen in the tops and roots is shown to be the greatest 2 weeks before blooming, considerable decreases occurring at each succeeding stage of growth. The largest amount of total nitrogen in the plants was found at full bloom, a decrease occurring at maturity.

The largest per cent of the total nitrogen was found in the roots when the blooms appeared, although the difference was not very great over the amount in the roots 2 weeks earlier. At full bloom a much smaller proportion was present in the roots and at maturity a small increase had occurred. On the average about 22 per cent of the total nitrogen was found in the roots.

TABLE 3

Clover in unsterilized, uninoculated Carrington loam

POT NUMBER	SAMPLING	NITROGEN IN SOIL	TOTAL NITROGEN IN POTS	NITROGEN IN PLANTS	NITROGEN IN SOIL AND PLANTS	NITROGEN IN POTS AT BEGINNING OF EXPERIMENT	GAIN IN NITROGEN IN POTS	AVERAGE NITROGEN FIXED PER POT	AVERAGE GAIN IN NITROGEN PER PLANT
		per cent	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*cgm.*
1	Two weeks before blooming...........	0.2678	10.5543	0.2208	10.7751	10.6449	0.1302		
2	Two weeks before blooming...........	0.2678	10.5543	0.2323	10.7866	10.6449	0.1417	0.1359	1.1
3	When blooms appeared.	0.2564	10.1050	1.4780	11.5830	10.6449	0.9381		
4	When blooms appeared.	0.2638	11.1136	1.6474	12.7610	11.3790	1.3820	1.1600	9.6
5	Full bloom............	0.2616	10.4284	2.2041	12.6325	10.7673	1.8652		
6	Full bloom............	0.2671	10.6477	1.3380	11.9857	10.7673	1.2184	1.5418	12.8
7	Mature................	0.2567	10.1168	1.6089	11.7257	10.6449	1.0808		
8	Mature................	0.2446				12.3579		1.0808	9.0

In table 3 appear the results of the nitrogen determinations on the soils before and after cropping and the calculations of the gains in nitrogen by the growth of the legume or the nitrogen fixed from the atmosphere, figured in grams per pot. The results are calculated as centigrams per plant and not in pounds per acre as the crop yields are so much greater than are ever obtained in the field, and as a consequence the increases in nitrogen are far greater than could occur in the field. Evidently the red clover bacteria were present in the soil, for a large fixation of nitrogen occurred and the plants were thoroughly inoculated.

No account is taken in these calculations of the nitrogen fixed by *Azotobacter* in these pots and in some of the experiments, as will be noted later, there was evidently some action of these non-symbiotic organisms. The process of azofication is of such common occurrence however, that without special precautions it could not be eliminated in these tests, particularly with-

out sterilizing the soil and as the amounts fixed are small (amounting to not more than 0.100 gm. per pot in special tests on the same soils) compared with those assimilated by the legumes, the process is not generally considered. It is apparent from these results that there was an increase in the amount of nitrogen fixed by the plants up to the period of full bloom but beyond that a decrease occurred. Only one pot being harvested at maturity, however, renders the results at that time uncertain and they should not be taken as conclusive. At full bloom the amount of nitrogen fixed amounted to 12.8 cgm. per plant, tops and roots, and 1.1 cgm. per plant, roots alone. These figures indicate to what extent the growth of an inoculated crop of red clover may enrich the soil in nitrogen when it is used as a green manure, or when it is removed for hay and only the roots remain in the soil. Calculating these gains in nitrogen in per cent of the total amount in the plants, the following figures are secured:

SAMPLING	NITROGEN IN PLANTS TAKEN FROM THE AIR
	per cent
Two weeks before blooming	60.0
When blooms appeared	74.3
Full bloom	87.1
Mature	67.1

At full bloom a very large proportion of the total nitrogen of the plants was taken from the air. Other results may show a still further increase at maturity or at least no decrease, and the decrease noted here was probably due to some unusual condition in the one pot which was carried to maturity.

It is interesting also to calculate the proportion of the nitrogen present in the tops only of the clover, which was fixed from the atmosphere. Utilizing the figures from table 2, which show the total nitrogen in the tops and roots, the following figures are secured:

SAMPLING	NITROGEN IN THE TOPS TAKEN FROM THE AIR
	per cent
Two weeks before blooming	79.2
When blooms appeared	98.9
Full bloom	100.0*
Mature	85.4

* Some of the nitrogen in the roots came from the air in this case and the actual figure here is greater than 100 per cent.

The proportion of nitrogen in the tops taken from the air increased up to full bloom and then decreased. In fact, at full bloom, all the nitrogen of the tops was taken from the atmosphere and a part of that in the roots also came from the air. The results secured at maturity were not definite and

hence conclusions are difficult, but the results of the series as a whole indicate that with inoculated red clover on this soil not only may all the nitrogen in the tops represent "new" nitrogen taken from the air, but the roots may also contain more than "old" nitrogen secured from the soil.

Series II

In this series clover was grown on Carrington loam, sterilized and inoculated. The weights in grams of the tops and roots of the plants, the percentage of nitrogen in the tops and roots and the total nitrogen in the tops, roots and plants are given in table 4. The average results from these figures, the percentage of the total plant growth in the tops and roots and the percentage of the total nitrogen of the plant in the tops and roots are shown in table 5.

It is evident from the figures in this table, first of all that the total weight of the plants increased up to maturity, the largest increase occurring between the stages of 2 weeks before blooming and when the blooms appeared. A large increase also was found from the latter period to the period of full bloom. In the case of the tops, the increases were much the same proportionately as of the entire plant but with the roots the increases were small after the first period. The largest percentage of plant growth in the roots was found when the blooms appeared, smaller proportions being present at the later stages of growth. This is in accord with the results obtained in the preceding series. The average proportion of the total plant growth in the roots at the four stages of growth was 30 per cent. This figure is slightly smaller than that secured in the previous series but checks very well with it.

The total nitrogen of the plants increased up to maturity but the greatest increases occurred between the first and second periods just as was noted in the case of the weights of the crop. In table 4 it is seen that the greatest percentage of nitrogen was found in both tops and roots at the first stage of growth. At subsequent periods the proportion decreased, the differences, however, being small. At the last two stages the greatest decrease occurred between the first period and the second. The largest percentage of the total nitrogen of the plants in the roots is shown in table 5 to be present at the first stage of growth, the amounts decreasing at the later periods, only 19.7 per cent being found in the roots at maturiy. The average proportion of the total nitrogen in the roots at all stages of growth was about 22 per cent, exactly the same figure as was obtained in the preceding series. The results thus far are therefore apparently very much the same on the Carrington loam whether unsterilized or sterilized and inoculated. The inoculating bacteria were evidently present in the soil and whether or not they were as effective as those introduced into the sterile soil cannot be determined on account of the sterilization. The sterilization of the soil, however, apparently did not cause any interference with the best growth of the crop, an effect which is frequently noted when soil is subjected to steam under pressure.

TABLE 4

Clover in sterilized, inoculated Carrington loam

POT NUMBER	WEIGHT OF						NITROGEN IN				TOTAL NITROGEN IN					
	Tops	Average	Roots	Average	Plants	Average	Tops	Average	Roots	Average	Tops	Average	Roots	Average	Plants	Average
	gm.	gm.	gm.	gm.	gm.	gm.	per cent	per cent	per cent	per cent	gm.	gm.	gm.	gm.	gm.	gm.
1	5.98		2.48		8.46		3.270		2.670		0.1955		0.0662		0.2617	
2	5.54	5.76	2.57	2.52	8.11	8.28	3.440	3.355	2.720	2.695	0.1905	0.1930	0.0699	0.0680	0.2604	0.2610
3	46.85		22.80		69.65		2.790		1.860		1.3071		0.4240		1.7311	
4	55.40	51.13	30.99	26.89	86.39	78.02	2.550	2.670	1.550	1.705	1.4127	1.3599	0.4803	0.4521	1.8930	1.8120
5	89.45		31.70		121.15		2.440		1.582		2.1826		0.5015		2.6841	
6	74.10	81.77	36.72	34.21	110.82	115.98	2.457	2.448	1.600	1.591	1.8206	2.0016	0.5875	0.5445	2.4081	2.5461
7	89.00		38.93		127.93		2.291		1.324		2.0389		0.5154		2.5543	
8	97.10	93.05	29.95	34.44	127.05	127.49	2.365	2.328	1.829	1.576	2.2964	2.1676	0.5478	0.5316	2.8442	2.6992

TABLE 5

Clover in sterilized, inoculated Carrington loam

SAMPLING	AVERAGE WEIGHT OF			PLANT GROWTH IN		AVERAGE TOTAL NITROGEN IN			TOTAL NITROGEN IN	
	Tops	Roots	Plants	Tops	Roots	Tops	Roots	Plants	Tops	Roots
	gm.	gm.	gm.	per cent	per cent	gm.	gm.	gm.	per cent	per cent
Two weeks before blooming	5.76	2.52	8.28	69.56	30.44	0.1930	0.0680	0.2610	73.94	26.06
When blooms appeared	51.13	26.89	78.02	65.53	34.47	1.3599	0.4521	1.8120	75.05	24.95
Full bloom	81.77	34.21	115.98	70.58	29.42	2.0016	0.5445	2.5461	78.61	21.39
Mature	93.05	34.44	127.49	72.98	27.02	2.1676	0.5316	2.6992	80.30	19.70

In table 6 appear the results of the nitrogen determinations on the soil before and after growth of the legume and the calculations showing the nitrogen fixed by the crop. The gain in nitrogen is calculated in grams per pot and as centigrams per plant. Examining this table there is found to be a gradually increasing fixation of nitrogen from the atmosphere at succeeding stages of growth. Thus at full bloom twice as much nitrogen was fixed as at the preceding stage, while at maturity a further increase is noted. The greatest gain apparently occurred between the second and third stages of growth. In the preceding series the greatest gain took place between the first and second stages. This difference may have been due to differences in the growth of the plants or to variations in the rate of inoculation. It is interesting to note

TABLE 6

Clover in sterilized, inoculated Carrington loam

POT NUMBER	SAMPLING	NITROGEN IN SOIL	TOTAL NITROGEN IN POTS	NITROGEN IN PLANTS	NITROGEN IN SOIL AND PLANTS	NITROGEN IN POTS AT BEGINNING OF EXPERIMENT	GAIN IN NITROGEN IN POTS	AVERAGE NITROGEN FIXED PER POT	AVERAGD GAIN IN NITROGEN PER PLANT
		per cent	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*cgm.*
1	Two weeks before blooming	0.2704	11.0242	0.2617	11.2859	11.0120	0.2739		
2	Two weeks before blooming	0.2685	10.9467	0.2604	11.2071	11.0120	0.1951	0.2345	1.9
3	When blooms appeared	0.2540	10.0104	1.7311	11.7415	10.6449	1.0966		
4	When blooms appeared	0.2468	11.7390	1.8930	13.6320	12.8473	0.7847	0.9406	7.8
5	Full bloom	0.2543	10.8286	2.6841	13.5127	11.5014	2.0113		
6	Full bloom	0.2524	10.6333	2.4081	13.0414	11.3790	1.6624	1.8368	15.3
7	Mature	0.2566	10.4616	2.5543	13.0159	11.0120	2.0039		
8	Mature	0.2519	10.3811	2.8442	13.2253	11.1343	2.0910	2.0474	17.0

that in this series the fixation increased up to maturity, thus indicating that in the preceding test, as was noted there, the results at maturity were probably abnormal. The actual amount of nitrogen fixed was greater on the sterilized inoculated soil in all but one case, which was at the second stage of growth. This may be taken to indicate that the organisms introduced into the sterilized soil proved more effective than those present in the unsterilized soil. The differences are not great, however, especially at the full-bloom stage, but they are great enough to be important if the value of the nitrogen fixed were figured on the basis of the market price of the element in commercial fertilizers.

The percentage of the total nitrogen in the plants taken from the air is shown in the following figures:

SAMPLING	NITROGEN IN PLANTS TAKEN FROM THE AIR
	per cent
Two weeks before blooming	89.8
When blooms appeared	51.9
Full bloom	72.1
Mature	75.8

The largest percentage fixation was found at the first stage. Following that period there was a decrease in the proportion fixed but at the later stages the percentages increased considerably. At maturity three-fourths of the nitrogen of the plants apparently came from the air. This amount is somewhat greater than that noted in the preceding test but the percentage fixed at full bloom is smaller than that in the other test and the same is true at the second stage of growth. These results probably indicate something of the differences which occur in the field when legumes are inoculated in different ways and may be a reflection of the rapidity or completeness with which the plants become inoculated. It is also quite possible that the sterilization exerted some influence on the rate of inoculation or on the rate at which nitrogen from the soil was supplied to the plants. At the later stages of growth, however, the greater efficiency of the organisms probably brought about the larger fixation and also the larger crop growth.

The percentage of the nitrogen in the tops of the crop, which came from the atmosphere is shown in the following figures:

SAMPLING	NITROGEN IN THE TOPS TAKEN FROM THE AIR
	per cent
Two weeks before blooming	100.0*
When blooms appeared	69.1
Full bloom	91.7
Mature	94.4

* Over 100 per cent.

At the first stage all the nitrogen in the tops was taken from the air and a part of that in the roots was secured from the air also. At the second stage only 69 per cent of the total amount in the tops came from the air. At the later samplings, however, almost all of the nitrogen was taken from the air. Again these variations may be due to the differences in rate of inoculation, rate of growth or efficiency of the organisms but it is evident that practically all of the nitrogen in the tops of the clover came from the atmosphere and the amount present in the roots evidently was taken from the soil. Small differences cannot be considered of great significance in this work, since such small amounts of nitrogen and such small variations in percentage are involved.

Series III

In series III alfalfa, was grown on Carrington loam unsterilized and uninoculated. Table 7 shows the weights of the tops and roots at the different stages of growth, the percentage of nitrogen in the tops and roots and the total nitrogen present in the tops, the roots and the whole plants. The averages of these figures are given in table 8 and calculations also are given showing the per cent of total plant growth present in the tops and roots and the per cent of the total nitrogen of the crop in the tops and roots. As was noted with the clover the crop increased in weight up to maturity, the greatest increase occurring between the first and second periods. A large gain also was found between the second and third periods, but from full bloom to maturity only a relatively small increase occurred. Very much the same increases as these are noted in the case of both the tops and the roots, and the increases at the different stages were quite similar with the two portions of the plants. Apparently with this crop the tops and roots develop at about the same rate. The only difference noted is at the second stage when the roots showed a greater increase than did the tops. The greatest percentage of the total weight of the plants was found in the roots at the second stage of growth while at later stages the figures were somewhat smaller. At the first stage only a small proportion of the plant was in the roots but the roots soon began to develop and made a very rapid growth before the appearance of blooms. On the average over 41 per cent of the plant was in the roots, and if the figures at the first stage are not included the average shows over 50 per cent of the crop present in the roots. These figures indicate that alfalfa has a larger proportion of roots to tops than does red clover.

From table 7 it will be seen that the greatest percentages of nitrogen were present both in the tops and roots at the first stage of growth. At later stages decreases occurred. In the case of the tops these decreases continued up to maturity but with the roots a slight increase occurred at the third period. This, however, was followed by a decrease at maturity. The total nitrogen in the plants increased up to full bloom and decreased slightly at maturity. The difference here was not great and probably should not be taken as conclusive. The largest percentage of the total nitrogen in the roots was found at maturity but the differences were not great after the second stage of growth. The largest gain of nitrogen in the roots occurred between the first and second stages. On the average over 35 per cent of the total nitrogen of the alfalfa was in the roots and if the figures at the first period are not included, 43 per cent is found in the roots. This is about twice as much as was found in the case of the clover in the two preceding series.

In table 9 are given the results of the nitrogen determinations and the calculations of the gain in nitrogen in the pots and per plant, or the nitrogen fixed by the alfalfa. Evidently the proper bacteria were present in the soil, for the crop became inoculated and a large fixation of nitrogen occurred.

TABLE 7

Alfalfa in unsterilized, uninoculated Carrington loam

POT NUMBER	WEIGHT OF						NITROGEN IN				TOTAL NITROGEN IN					
	Tops	Average	Roots	Average	Plants	Average	Tops	Average	Roots	Average	Tops	Average	Roots	Average	Plants	Average
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*per cent*	*per cent*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	3.35		0.49		3.84		3.540		3.180		0.1186		0.0156		0.1342	
2	3.14	3.24	0.60	0.54	3.74	3.78	3.370	3.455	3.040	3.110	0.1058	0.1122	0.0182	0.0169	0.1240	0.1291
3	21.05		27.75		48.80		3.097		1.834		0.6519		0.5089		1.1608	
4	22.25	21.65	22.95	25.35	45.20	47.00	3.050	3.073	1.917	1.875	0.6786	0.6652	0.4400	0.4745	1.1186	1.1397
5	33.70		37.20		70.90		2.803		2.290		0.9446		0.8519		1.7965	
6	53.90	43.80	47.50	42.35	101.40	86.15	2.426	2.614	2.018	2.154	1.3076	1.1261	0.9586	0.9052	2.2662	2.0313
7	48.10		48.00		96.10		2.471		1.780		1.1886		0.8544		2.0430	
8	46.00	47.05	46.40	47.20	92.40	94.25	2.036	2.253	1.897	1.838	0.9366	1.0626	0.8802	0.8673	1.8168	1.9299

TABLE 8

Alfalfa in unsterilized, uninoculated Carrington loam

SAMPLING	AVERAGE WEIGHT OF			PLANT GROWTH IN		AVERAGE TOTAL OF NITROGEN IN			TOTAL NITROGEN IN	
	Tops	Roots	Plants	Tops	Roots	Tops	Roots	Plants	Tops	Roots
	gm.	*gm.*	*gm.*	*per cent*	*per cent*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*
Two weeks before blooming	3.24	0.54	3.78	85.71	14.29	0.1122	0.0169	0.1291	86.91	13.09
When blooms appeared	21.65	25.35	47.00	46.06	53.94	0.6652	0.4745	1.1397	58.36	41.64
Full bloom	43.80	42.35	86.15	50.84	49.16	1.1261	0.9052	2.0313	55.43	44.57
Mature	47.05	47.20	94.25	49.92	50.08	1.0626	0.8673	1.9299	55.06	44.94

TABLE 9

Alfalfa in unsterilized, uninoculated Carrington loam

POT NUMBER	SAMPLING	NITROGEN IN SOIL	TOTAL NITROGEN IN POTS	NITROGEN IN PLANTS	NITROGEN IN SOIL AND PLANTS	NITROGEN IN POTS AT BEGINNING OF EXPERIMENT	GAIN IN NITROGEN IN POTS	AVERAGE NITROGEN FIXED PER POT	AVERAGE GAIN IN NITROGEN PER PLANT
		per cent	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*cgm.*
1	Two weeks before blooming	0.2696	10.8695	0.1342	11.0037	10.8896	0.1141		
2	Two weeks before blooming	0.2699	12.3487	0.1240	12.4727	12.3579	0.1148	0.1144	1.2
3	When blooms appeared	0.2572	10.4860	1.1608	11.6468	11.0120	0.6348		
4	When blooms appeared	0.2555	7.8707	1.1186	8.9893	8.3199	0.6694	0.6521	7.2
5	Full bloom	0.2528	11.2228	1.7965	13.0193	11.9908	2.0285		
6	Full bloom	0.2548	10.3728	2.2662	12.6490	10.8896	1.7594	1.8938	21.0
7	Mature	0.2789	11.2444	2.0430	13.2874	10.8896	2.3978		
8	Mature	0.2778	11.4517	1.8168	13.2685	11.1343	2.0942	2.2460	24.9

An increase in nitrogen fixed was noted at each successive stage of growth, at maturity 24.9 cgm. of nitrogen per plant being obtained from the atmosphere. The greatest increase occurred between the second and third stages of growth. With the roots alone the fixation amounted to 12.4 cgm. per plant.

The percentage of the total nitrogen in the plants taken from the atmosphere is shown in the following figures:

SAMPLING	NITROGEN IN PLANTS TAKEN FROM THE AIR
	per cent
Two weeks before blooming	88.6
When blooms appeared	57.2
Full bloom	93.2
Mature	100.0*

* Over 100 per cent.

The largest percentage of total nitrogen taken from the air probably was in the plants at maturity. The figures, however, are uncertain, inasmuch as there was evidently some fixation of nitrogen by non-symbiotic bacteria. The soil showed a gain in nitrogen apart from that fixed by the legumes and that gain probably came from the action of the azofiers. At full bloom, however, almost all of the nitrogen of the plants (tops and roots) came from the atmosphere. The smallest proportion of nitrogen from the air was taken by the plants at the second stage of growth, while at the first stage a large amount came from the atmosphere.

Calculating the percentage of the nitrogen in the tops taken from the air, the following figures are obtained:

SAMPLING	NITROGEN IN THE TOPS TAKEN FROM THE AIR
	per cent
Two weeks before blooming	100.0*
When blooms appeared	98.0
Full bloom	100.0*
Mature	

* Over 100 per cent.

At every stage of growth practically the entire amount of nitrogen in the tops was fixed from the air and at the last two stages the amount in the roots also was very largely secured from the atmosphere. The interference of the non-symbiotic organisms makes the conclusions difficult but at least it may be said that the entire amount in the tops was taken from the air and probably a portion of that in the roots was secured from the same source.

Series IV

In this series alfalfa was grown on Carrington loam sterilized and then inoculated. The weights of the tops and roots and the nitrogen present in each are shown in table 10. The averages from the duplicate pots and the calculations of the percentages of plant growth in the tops and roots and of the proportion of the total nitrogen in the tops and roots are given in table 11.

From the figures given in the latter table it is apparent that the weight of the plants increased at each stage of growth, the largest increase occurring at the third stage. The gain from the first to the second stage, however, was almost as great. This is in accord with the results secured on the unsterilized soil. As in that case also the tops and roots increased in a very similar way, the largest increase in both cases occurring between the second and third stages. The increase in both tops and roots was small from full bloom to maturity. In this series the gain at the second stage in the roots was not as great as that in the tops, differing from the results of the preceding test. This difference may be due to the difference in the soil conditions brought about by the sterilization, possibly a bacterial variation, but the figures are not sufficiently far apart to warrant conclusions.

The largest percentage of the total nitrogen of the plants was found in the roots at maturity just as in the preceding case. The greatest increase also, as noted before, occurred from the first to the second stages of growth. A slightly smaller percentage was in the roots at maturity, but the difference was not great. The average percentage in the roots at all stages was 34, as against 35 per cent in the preceding test, and if the figures obtained 2 weeks before blooming are not included the average is 39 per cent against 43 per cent. These differences again may be due to the soil conditions. From table 10 it appears that the percentage of nitrogen in the tops decreased at each

TABLE 10

Alfalfa in sterilized, inoculated Carrington loam

POT NUMBER	WEIGHT OF						NITROGEN IN				TOTAL NITROGEN IN					
	Tops	Average	Roots	Average	Plants	Average	Tops	Average	Roots	Average	Tops	Average	Roots	Average	Plants	Average
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*per cent*	*per cent*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	2.10		0.57		2.67		3.710		3.600		0.0779		0.0205		0.0984	
2	3.72	2.91	0.45	0.51	4.17	3.42	3.130	3.420	3.770	3.685	0.1164	0.0972	0.0170	0.0187	0.1334	0.1159
3	27.32		19.55		46.87		2.190		1.720		0.5983		0.3363		0.9346	
4	23.72	25.52	22.15	20.85	45.87	46.37	2.900	2.545	1.750	1.735	0.6879	0.6431	0.3876	0.3619	1.0755	1.0050
5	52.10		46.80		98.90		2.775		1.927		1.4457		0.9018		2.3475	
6	46.00	49.05	39.21	43.00	85.21	92.05	2.191	2.483	1.907	1.917	1.0078	1.2267	0.7477	0.8248	1.7555	2.0515
7	54.75		52.03		106.78		2.129		1.870		1.1656		0.9730		2.1386	
8	54.20	54.47	50.75	51.39	104.95	105.86	2.517	2.323	1.774	1.822	1.3642	1.2649	0.9003	0.9366	2.2645	2.2015

TABLE 11

Alfalfa in sterilized, inoculated Carrington loam

SAMPLING	AVERAGE WEIGHT OF			PLANT GROWTH IN		AVERAGE TOTAL NITROGEN IN			TOTAL NITROGEN IN	
	Tops	Roots	Plants	Tops	Roots	Tops	Roots	Plants	Tops	Roots
	gm.	*gm.*	*gm.*	*per cent*	*per cent*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*
Two weeks before blooming	2.91	0.51	3.42	85.09	14.91	0.0972	0.0187	0.1159	83.86	16.14
When blooms appeared	25.52	20.85	46.37	55.04	44.96	0.6431	0.3619	1.0050	63.99	36.01
Full bloom	49.05	43.00	92.05	53.28	46.72	1.2267	0.8248	2.0515	59.79	40.21
Mature	54.47	51.39	105.86	51.45	48.55	1.2649	0.9366	2.2015	57.45	42.55

stage of growth, the greatest decrease occurring at the second stage. With the roots the results vary somewhat, a large decrease occurring at the second stage, this being followed by an increase at the third period and a slight decrease at maturity. These results agree exactly with those secured in the preceding series.

In table 12 appear the results of the nitrogen determinations on the soil and the calculations of the nitrogen fixed by the crop.

TABLE 12

Alfalfa in sterilized inoculated Carrington loam

POT NUMBER	SAMPLING	NITROGEN IN SOIL	TOTAL NITROGEN IN POTS	NITROGEN IN PLANTS	NITROGEN IN SOIL AND PLANTS	NITROGEN IN POTS AT BEGINNING OF EXPERIMENT	GAIN IN NITROGEN IN POTS	AVERAGE NITROGEN FIXED PER POT	AVERAGE GAIN IN NITROGEN PER PLANT
		per cent	gm.	gm.	gm.	gm.	gm.	gm.	cgm.
1	Two weeks before blooming	0.2678	10.9182	0.0984	11.0166	11.0120	0.0046		
2	Two weeks before blooming	0.2730	10.7592	0.1334	10.8926	10.6449	0.2477	0.1261	1.4
3	When blooms appeared	0.2595	11.6378	0.9346	12.5724	12.1132	0.4592		
4	When booms appeared	0.2542	11.0546	1.0755	12.1301	11.7461	0.3840	0.4216	4.6
5	Full bloom	0.2548	11.1962	2.3475	13.5437	11.8685	1.6752		
6	Full bloom	0.2602	10.3726	1.7555	12.1281	10.7673	1.3608	1.5180	16.8
7	Mature	0.2582	10.4098	2.1386	12.5484	10.8896	1.6588		
8	Mature	0.2637	10.3927	2.2645	12.6572	10.6449	2.0123	1.8355	20.3

The fixation of nitrogen here was somewhat less than that found in the unsterilized Carrington loam. Increases in fixation were noted up to maturity, the greatest increase occurring at the third stage of growth. Inoculation was thorough in this case and the nitrogen fixed shows quite accurately the ability of the legume to utilize the nitrogen of the atmosphere.

Comparisons of the fixation on this soil sterilized and inoculated with that occurring when the soil was not sterilized are not possible, on account of the possibility of somewhat greater action of the azofiers in the latter case but it would seem that the amount of nitrogen fixed by the legumes was about the same in the two cases.

Calculations of the percentage of the total nitrogen in the plants taken from the air are shown below:

SAMPLING	NITROGEN IN THE PLANTS TAKEN FROM THE AIR
	per cent
Two weeks before blooming	
When blooms appeared	41.9
Full bloom	73.9
Mature	83.3

Two weeks before blooming the entire nitrogen supply seems to have been taken from the air, but as the results are not satisfactory, the duplicate pots not agreeing, conclusions should not be drawn. At the later stages increasingly large proportions of the nitrogen came from the air until at maturity 83.3 per cent was secured in this way. The largest increase occurred between the second and third stages. Comparisons with the results on the unsterilized soil show that a somewhat smaller percentage of the total nitrogen in the plants came from the air, which may be due to a fixation of nitrogen by the azofiers in the unsterilized soil.

The percentage of the nitrogen in the tops taken from the atmosphere is shown in the following figures:

SAMPLING	NITROGEN IN THE TOPS TAKEN FROM THE AIR
	per cent
Two weeks before blooming	
When blooms appeared	65.5
Full bloom	100.0*
Mature	100.0*

* Over 100 per cent.

At the first stage of growth the results are unsatisfactory, as noted earlier. At the second stage 65 per cent of the nitrogen in the tops came from the air. This compares with 100 per cent obtained in the preceding test. At the later stages all the nitrogen of the tops was taken from the atmosphere and a part of that in the roots was secured from the same source. Similar results were secured also in the test on the unsterilized soil. It seems to be evident from these results with inoculated alfalfa that all the nitrogen in the tops came from the air and in some cases a *part of that in the roots also was taken from the atmosphere.*

Series V

In this series clover was grown on unsterilized, uninoculated Miami fine sandy loam. The results in table 13 show the weights of the tops, roots and crop at the various stages of growth, the percentage of nitrogen in the tops and roots and the total nitrogen present in each. The crop was not secured on one of the pots 2 weeks before blooming and therefore the results have no check at that period. The average weights and the average nitrogen content of the tops, roots and plants are given in table 14, and the calculations of the percentage of plant growth in the tops and roots and of the percentage of total nitrogen in the tops and roots are given in the same table.

The results in this table show first of all large increases in crop growth at each succeeding stage. The largest increase occurred between the first and second periods. This was true also for the tops and roots. There was a larger gain in the case of the tops than with the roots at the second period,

TABLE 13

Clover in unsterilized, uninoculated Miami fine sandy loam

POT NUMBER	WEIGHT OF						NITROGEN IN				TOTAL NITROGEN IN					
	Tops	Average	Roots	Average	Plants	Average	Tops	Average	Roots	Average	Tops	Average	Roots	Average	Plants	Average
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*per cent*	*per cent*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1																
2	1.65	1.65	0.60	0.60	2.25	2.25	3.23	3.23	3.02	3.02	0.0533	0.0533	0.0181	0.0181	0.0714	0.0714
3	39.95		27.55		67.50		2.55		1.60		1.0187		0.4408		1.4595	
4	35.95	37.95	21.12	24.33	57.07	62.28	2.31	2.43	1.91	1.75	0.8304	0.9245	0.4034	0.4221	1.2338	1.3466
5	44.95		28.45		73.40		2.45		1.68		1.1012		0.4779		1.5791	
6	52.55	48.75	42.95	35.70	95.50	84.45	2.25	2.35	1.50	1.59	1.1823	1.1417	0.6442	0.5611	1.8265	1.7028
7	63.10		39.35		102.45		2.12		1.61		1.3377		0.6335		1.9712	
8	59.20	61.15	45.35	42.35	104.55	103.50	2.17	2.14	1.67	1.64	1.2846	1.3111	0.7573	0.6954	2.0419	2.0065

TABLE 14

Clover in unsterilized, uninoculated Miami fine sandy loam

SAMPLING	AVERAGE WEIGHT OF			PLANT GROWTH IN		AVERAGE TOTAL NITROGEN IN			TOTAL NITROGEN IN	
	Tops	Roots	Plants	Tops	Roots	Tops	Roots	Plants	Tops	Roots
	gm.	*gm.*	*gm.*	*per cent*	*per cent*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*
Two weeks before blooming	1.65	0.60	2.25	73.33	26.67	0.0533	0.0181	0.0714	74.65	25.35
When blooms appeared	37.95	24.33	62.28	60.95	39.05	0.9245	0.4221	1.3466	68.65	31.35
Full bloom	48.75	35.70	84.45	57.72	42.28	1.1417	0.5611	1.7028	67.04	32.96
Mature	61.15	42.35	103.50	59.08	40.92	1.3111	0.6954	2.0065	65.34	34.66

and at the fourth period the tops increased more than did the roots. The largest percentage of the total plant growth in the roots was found at full bloom, a decrease ocurring at maturity. The smallest proportion was in the roots 2 weeks before blooming and a large increase had occurred when the blooms appeared. The average proportion of the plants in the roots was 36 per cent, which is somewhat higher than that found when clover was grown on unsterilized Carrington loam (31 per cent). This difference may be due to the varying soil conditions in these two types.

The percentage of the total nitrogen in the roots increased up to maturity, the increase being the greatest at the second period. The average percentage was 31 as against 22 per cent in the case of the Carrington loam. The differences in the soils used evidently influenced the relative development of tops and roots, and also their nitrogen content. The percentage of nitrogen in the tops

TABLE 15

Clover in unsterilized, uninoculated Miami fine sandy loam

POT NUMBER	SAMPLING	NITROGEN IN SOIL	TOTAL NITROGEN IN POT	NITROGEN IN PLANTS	NITROGEN IN SOIL AND PLANTS	NITROGEN IN POTS AT BEGINNING OF EXPERIMENT	GAIN IN NITROGEN IN POTS	AVERAGE NITROGEN FIXED PER POT	AVERAGE GAIN IN NITROGEN PER PLANT
		per cent	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*cgm.*
1	Two weeks before blooming								
2	Two weeks before blooming	0.1368	5.8872	0.0714	5.9586	5.8226	0.1360	0.1360	1.1
3	When blooms appeared	0.1340	5.3418	1.4595	6.8013	5.3936	1.4077		
4	When blooms appeared	0.1346	5.4876	1.2338	6.7214	5.5162	1.2052	1.3064	10.8
5	Full bloom	0.1322	5.7689	1.5791	7.3480	6.0065	1.3415		
6	Full bloom	0.1304	5.7299	1.8265	7.5564	5.9452	1.6112	1.4763	12.3
7	Mature	0.1318	5.7842	1.9712	7.7554	5.9452	1.8102		
8	Mature	0.1345	4.7524	2.0419	6.7943	4.7807	2.0136	1.9119	15.9

and roots as shown in table 13 decreased at each stage of growth, the largest decrease occurring at the second period. The variations at the later samplings were not very large nor definite.

In table 15 are given the results of the nitrogen determinations on the soils before and after growth of the legumes, and the gain in nitrogen expressed as grams per pot and as pounds per acre.

It is evident that the clover became thoroughly inoculated on this soil and a large fixation of nitrogen from the atmosphere took place. The largest fixation occurred at maturity, increases occurring at each period, the largest gains being from the first to the second and from the third to the fourth periods. At maturity 15.9 cgm. of nitrogen were fixed per plant, tops and roots, and 5.0 cgm. per plant, roots alone. These amounts are much greater than those secured on the unsterilized Carrington loam.

The percentages of the total plant nitrogen taken from the air are given below:

SAMPLING	NITROGEN IN PLANTS TAKEN FROM THE AIR
	per cent
Two weeks before blooming	
When blooms appeared	97.0
Full bloom	86.6
Mature	95.2

The results at the first period are abnormal and were not duplicated, and hence are not included here. At the later periods the plants took most of their nitrogen from the air—the figures being very much larger than on the Carrington loam. Calculating the percentage of nitrogen in the tops taken from the atmosphere it is found that the total amount of nitrogen in the tops was secured from the air at all stages of growth and a large part of that in the roots was similarly secured. There was probably some non-symbiotic nitrogen fixation in this soil and the results are modified therefore to a small but unknown extent. Apparently, however, there was much difference in the results on this soil from those on the Carrington loam unsterilized. A much larger fixation of nitrogen occurred at maturity and a somewhat larger proportion of the nitrogen in the plants came from the air.

Series VI

In this series clover was grown on sterilized, inoculated Miami fine sandy loam. The results in table 16 show the weights of the tops and roots, the percentage of nitrogen present in each and the total nitrogen content of the tops, roots and plants. The average results and the determinations of the percentage of plant growth in the tops and roots and the percentage of total nitrogen in tops and roots are given in table 17. From the latter table it appears that the largest increase in total average weight of the plants, occurred between the first and second periods. At the later periods the increases were not so great, the smallest increase occurring at the last stage. The roots increased in weight somewhat more than the tops at the first period, but made little further gain. The tops, on the other hand, made a considerable increase at the third period. No further increase occurred at the fourth period.

The largest percentage of total plant growth in the roots was found at the second period, although there was little difference from the first. At the later periods there was a considerable decrease in the proportion of the plants in the roots. These results are somewhat different from those secured in the unsterilized, uninoculated Miami fine sandy loam in the preceding series, and it would seem that where the soil was sterilized and inoculated the roots developed much more rapidly. The difference may have been due either to the chemical difference in the soil brought about by the sterilization or to the difference in the bacterial factor or the thoroughness and rate of inoculation.

TABLE 16

Clover in sterilized, inoculated Miami fine sandy loam

POT NUMBER	WEIGHT OF						NITROGEN IN				TOTAL NITROGEN IN					
	Tops	Average	Roots	Average	Plants	Average	Tops	Average	Roots	Average	Tops	Average	Roots	Average	Plants	Average
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*per cent*	*per cent*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	1.64		1.25		2.89		2.930		3.720		0.0481		0.0465		0.0946	
2	0.98	1.31	1.53	1.39	2.51	2.70	3.180	3.055	2.470	3.095	0.0312	0.0396	0.0378	0.0422	0.0790	0.0818
3	21.25		17.80		39.05		2.147		1.550		0.4562		0.2759		0.7321	
4	16.25	18.75	22.70	20.25	38.95	39.00	2.211	2.179	1.170	1.360	0.3593	0.4077	0.2656	0.2708	0.6249	0.6785
5	41.00		22.10		63.10		2.078		1.440		0.8520		0.3182		1.1702	
6	27.50	34.25	20.90	21.50	48.40	55.75	2.231	2.154	1.284	1.362	0.6135	0.7327	0.2684	0.2933	0.8819	1.0260
7	34.10		13.95		48.05		2.064		1.521		0.7038		0.2122		0.9160	
8	33.90	34.00	18.44	16.19	52.34	50.19	1.888	1.976	1.568	1.544	0.6400	0.6719	0.2891	0.2506	0.9291	0.9225

TABLE 17

Clover in sterilized, inoculated Miami fine sandy loam

SAMPLING	AVERAGE WEIGHT OF			PLANT GROWTH IN		AVERAGE TOTAL NITROGEN IN			TOTAL NITROGEN IN	
	Tops	Roots	Plants	Tops	Roots	Tops	Roots	Plants	Tops	Roots
	gm.	*gm.*	gm.	*per cent*	*per cent*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*
Two weeks before blooming	1.31	1.39	2.70	48.51	51.49	0.0396	0.0422	0.0818	48.41	51.59
When blooms appeared	18.75	20.25	39.00	48.07	51.93	0.4077	0.2708	0.6785	60.09	39.91
Full bloom	34.25	21.50	55.75	61.43	38.57	0.7327	0.2933	1.0260	71.41	28.59
Mature	34.00	16.19	50.19	67.74	32.26	0.6719	0.2506	0.9225	72.83	27.17

The average percentage of plant growth in the roots was 43 against 39 per cent on the unsterilized soil. On the Carrington loam the corresponding percentages were 30 and 31. Hence it seems evident that the percentage of total plant growth was greater in the roots on the Miami fine sandy loam whether sterilized or not. This difference must be attributed to the variations in soil conditions, and these variations may be in any one factor or in several. It is quite impossible to do more than speculate as to the cause, but it seems likely that the difference in nitrogen and organic matter may be the chief factor responsible.

The percentage of total nitrogen in the roots decreased at every period, being the greatest at the first and smallest at the last. This is exactly the reverse of the results on the unsterilized soil, where an increase was noted up

TABLE 18

Clover in sterilized, inoculated Miami fine sandy loam

POT NUMBER	SAMPLING	NITROGEN IN SOIL	TOTAL NITROGEN IN POTS	NITROGEN IN PLANTS	NITROGEN IN SOIL AND PLANTS	NITROGEN IN POTS AT BEGINNING OF EXPERIMENT	GAIN IN NITROGEN IN POTS	AVERAGE NITROGEN FIXED PER POT	AVERAGE GAIN IN NITROGEN PER PLANT
		per cent	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*cgm.*
1	Two weeks before blooming	0.1351	5.9976	0.0946	6.0922	6.0065	0.0857		
2	Two weeks before blooming	0.1440	6.1318	0.0790	6.2108	5.6613	0.5495	0.3126	2.6
3	When blooms appeared	0.1344	6.2101	0.7321	6.9422	6.2144	0.7278		
4	When blooms appeared	0.1347	5.7358	0.6249	6.3607	5.6613	0.6994	0.7136	5.8
5	Full bloom	0.1263	5.5497	1.1702	6.7199	5.9452	0.7747		
6	Full bloom	0.1294	5.5687	0.8819	6.4506	5.8226	0.6280	0.7013	5.9
7	Mature	0.1226	5.7204	0.9160	6.6364	6.3130	0.3234		
8	Mature	0.1269	5.1737	0.9291	6.1028	5.5162	0.5866	0.4550	3.8

to maturity. Again this difference may have been due to the changed soil conditions, chemical or bacterial, or both, in the sterilized soil. Similar results were secured on the Carrington loam both when unsterilized and when sterilized and inoculated, and hence the cause of the difference in results in the unsterilized Miami fine sandy loam must have been due to the effects of the sterilization on that soil.

From the results of this series the average percentage of total nitrogen in the roots was 36 against 31 per cent in the unsterilized soil. On the Carrington loam the average percentage was 22 in both series. Thus it would seem that on the Miami fine sandy loam, a larger percentage of the total nitrogen of the plants was in the roots—on the average. This is in accord with the total-weight results and may be due to the difference in the nitrogen and organic matter in the soils.

In table 18 appear the results of the nitrogen determinations on the soils before and after cropping, and the calculations of the nitrogen gain in centigrams per plant. At the first period there was evidently some abnormality in the soil conditions in the duplicate pots, as they do not agree closely and the average nitrogen gain is so large that it indicates the possibility of the accidental introduction of azofiers and vigorous action by them. At the second period there was a fixation of 5.9 cgm. per plant by the clover, and of 2.5 cgm. per plant for the roots alone. At the two latter stages, however, the amount of nitrogen fixed was not increased and the largest fixation is recorded at the second and third periods. This is quite different from the results on the unsterilized soil in the preceding series where the fixation increased up to the last period when 15.9 cgm. per plant was found to be taken from the air. On the Carrington loam much larger amounts were fixed and increases were noted up to maturity. The soil differences and the effect of sterilization must be held responsible for these results on the Miami fine sandy loam. The inoculation was quite complete but it may be noted that the plants were much smaller on the sterilized soil and this may have been due to some physiological effect on the crop from products formed in the sterilization. The reverse was true on the Carrington loam and in that case evidently no injurious compounds were formed in the soil when it was sterilized.

The following figures give the percentage of the total nitrogen in the plants taken from the air:

SAMPLING	NITROGEN IN PLANTS TAKEN FROM THE AIR
	per cent
Two weeks before blooming	
When blooms appeared	100.0*
Full bloom	68.3
Mature	49.3

* Over 100 per cent.

The results at the first period were so abnormal that they are not given but the indications are that 100 per cent of the nitrogen was taken from the air. The same is true at the second period. At the later dates smaller percentages were found. These results are the reverse of those in the preceding series where practically all of the nitrogen came from the air. The sterilization evidently influenced the extent and efficiency of the inoculation.

In the table given below, appear the calculations of the percentage of the nitrogen in the tops taken from the atmosphere:

SAMPLING	NITROGEN IN THE TOPS TAKEN FROM THE AIR
	per cent
Two weeks before blooming	
When blooms appeared	100.0*
Full bloom	95.7
Mature	67.7

* Over 100 per cent.

At the first two stages practically all of the nitrogen of the plants seemed to come from the air and hence at those periods the total quantity in the tops was secured from the atmosphere. At the third period about the same result was noted, but at maturity only 67 per cent of the amount of nitrogen in the tops came from the air, the remainder coming from the soil. The crop reduction at the fourth stage on this soil is accompanied by a reduction in nitrogen fixed due to decreased thoroughness or efficiency in inoculation, or perhaps to increased assimilable nitrogen production from the sterilization.

Series VII

In this series, alfalfa was grown on unsterilized, uninoculated Miami fine sandy loam. The weights of the crop (tops and roots) the percentage of nitrogen in the tops and roots and in the total crop are given in table 19. The average results and the determinations of the percentages of total plant growth in the tops and roots and of total nitrogen in the tops and roots are given in table 20.

The total weight of the alfalfa increased at each period, the greatest increase occurring at the second period. Large gains were found at the two later periods, however. The largest percentage of the total plant growth was in the roots at the last period, a large increase being noted at the second period and small increases at each succeeding period. These results are very closely in accord with those secured on the unsterilized Carrington loam except that in this case increases in the percentage of plant growth in the roots were much more regular. The total nitrogen increased in the plants up to maturity and the percentage of the total amount in the crops which was in the roots increased similarly, the largest percentage being found at maturity. This agrees very well with the results on the Carrington loam. The average figures for the soil used in this series was 38 per cent, and this compares with 35 per cent in the case of the Carrington loam. The soil differences undoubtedly influenced the proportion of the total nitrogen of the plants which occurred in the roots.

Table 21 gives the results of the nitrogen determinations and the average gains in nitrogen at the various stages of growth. Apparently the inoculation of the alfalfa was quite efficient and the amounts of nitrogen fixed were considerable. The fixation increased up to maturity, the greatest changes occur-

TABLE 19

Alfalfa in unsterilized, uninoculated Miami fine sandy loam

POT NUMBER	WEIGHT OF						NITROGEN IN				TOTAL NITROGEN IN					
	Tops	Average	Roots	Average	Plants	Average	Tops	Average	Roots	Average	Tops	Average	Roots	Average	Plants	Average
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*per cent*	*per cent*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	1.84		0.60		2.44		3.83		3.27		0.0705		0.0196		0.0901	
2	1.61	1.72	0.52	0.56	2.13	2.28	3.62	3.72	3.30	3.28	0.0583	0.0644	0.0172	0.0184	0.0755	0.0828
3	29.80		18.75		48.55		2.38		1.55		0.7092		0.2906		0.9998	
4	21.40	25.60	27.10	22.92	48.50	48.52	2.37	2.37	1.91	1.73	0.5071	0.6081	0.5176	0.4041	1.0247	1.0122
5	34.10		25.00		59.10		2.44		1.54		0.8320		0.3850		1.2170	
6	27.15	30.62	41.45	33.23	68.60	63.85	2.19	2.31	1.97	1.70	0.5945	0.7132	0.8165	0.6008	1.4110	1.3140
7	36.00		44.45		80.45		2.35		1.75		0.8460		0.7778		1.6238	
8	36.10	36.05	45.95	45.20	82.05	81.25	2.36	2.35	1.70	1.72	0.8520	0.8490	0.7811	0.7794	1 6331	1.6284

TABLE 20

Alfalfa in unsterilized, uninoculated Miami fine sandy loam

SAMPLING	AVERAGE WEIGHT OF			PLANT GROWTH IN		AVERAGE TOTAL NITROGEN IN			TOTAL NITROGEN IN	
	Tops	Roots	Plants	Tops	Roots	Tops	Roots	Plants	Tops	Roots
	gm.	*gm.*	*gm.*	*per cent*	*per cent*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*
Two weeks before blooming	1.72	0.56	2.28	75.43	24.57	0.0644	0.0184	0.0828	77.77	22.23
When blooms appeared	25.60	22.92	48.52	52.76	47.24	0.6081	0.4041	1.0122	60.07	39.93
Full bloom	30.62	33.23	63.85	47.95	52.05	0.7132	0.6008	1.3140	54.27	45.73
Mature	36.05	45.20	81.25	44.37	55.63	0.8490	0.7794	1.6284	52.13	47.87

ring at the second and third periods. Only a comparatively small increase was noted at the fourth period. The fixation per plant was less than on the Carrington loam at the third and fourth stages, and this was probably a difference due to soil conditions.

TABLE 21

Alfalfa in unsterilized, uninoculated Miami fine sandy loam

POT NUMBER	SAMPLING	NITROGEN IN SOIL	TOTAL NITROGEN IN POT	NITROGEN IN PLANTS	NITROGEN IN SOIL AND PLANTS	NITROGEN IN POTS AT BEGINNING OF EXPERIMENT	GAIN IN NITROGEN IN POTS	AVERAGE NITROGEN FIXED PER POT	AVERAGE GAIN IN NITROGEN PER PLANT
		per cent	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*cgm.*
1	Two weeks before blooming	0.1342	5.4105	0.0901	5.5006	5.4549	0.0457		
2	Two weeks before blooming	0.1348	5.8622	0.0755	5.9377	5.8839	0.0538	0.0497	0.5
3	When blooms appeared	0.1313	5.8884	0.9998	6.8882	6.0678	0.8204		
4	When blooms appeared	0.1170	4.9417	1.0247	5.9664	5.4549	0.5115	0.6659	7.3
5	Full bloom	0.1376	5.4853	1.2170	6.7023	5.3936	1.3087		
6	Full bloom	0.1314	5.2381	1.4110	6.6491	5.3936	1.2555	1.2821	14.2
7	Mature	0.1319	5.6763	1.6238	7.3001	5.8226	1.4775		
8	Mature	0.1343	5.7796	1.6331	7.4127	5.8226	1.5901	1.5338	17.0

In the following table results are given showing the percentage of the total nitrogen in the plants, taken from the atmosphere:

SAMPLING	NITROGEN IN PLANTS TAKEN FROM THE AIR
	per cent
Two weeks before blooming	60.0
When blooms appeared	65.7
Full bloom	97.5
Mature	94.2

The largest percentage of total nitrogen in the plants was taken from the air at the last periods, almost the full amount coming in that way. There is no evidence of non-symbiotic fixation of nitrogen but even if it is assumed that some has occurred, most of the nitrogen in the plants at full bloom and at maturity must have been taken from the air.

At the earlier stages the proportion of nitrogen from the air was much lower. These results are quite in agreement with those secured with the alfalfa on the Carrington loam, where at maturity the total amount of nitrogen in the plants seemed to come from the air.

The calculations of the proportion of nitrogen in the tops taken from the air show that at the last three stages of growth all the nitrogen in the tops

came from the air and at the first period over three-fourths (77.1 per cent) was secured in that way. On the Carrington loam unsterilized, the total amount of nitrogen in the tops came from the air at every period, indicating the more rapid fixation of nitrogen in that soil. The results on the two soil types agree very well in showing the large amount of nitrogen which may be assimilated from the atmosphere. Even disregarding the possibility of non-symbiotic fixation of nitrogen which apparently occurred at least in the Carrington loam, it would seem that more than the nitrogen in the tops was taken from the air and at least a part of that in the roots was assimilated from the atmosphere.

Unfortunately, this series cannot be checked on sterilized inoculated soil on account of the failure of the crop under these conditions, perhaps due to physiological action which was noted in the case of the clover, an injurious effect of the sterilization.

DISCUSSION

It is quite impossible, of course, to draw any very definite or broadly applicable conclusions from the foregoing experiments. The results which have been secured must be considered applicable only to the two crops, red clover and alfalfa, and to the two soil types, Carrington loam and Miami fine sandy loam. They are also probably influenced to some extent by the fact that they were secured under greenhouse conditions and field results would not necessarily be exactly the same. They are indicative, however, of what may be expected in the field, and at least they serve to call attention to the variation in the proportion of plant growth of two legumes in tops and roots at different stages of growth and the differences in nitrogen content in tops and roots at the same stages, and under different soil conditions.

They indicate also how great the fixation of nitrogen from the atmosphere by individual leguminous plants may be under certain conditions. This throws some light on the question of how far legumes may be depended upon to keep up the nitrogen content of the soil, provided they are properly handled. In short, the data provide some evidence in support of previous assumptions, and show the impossibility of making definite statements now for all conditions. Further work along this line under different soil conditions is very desirable and the careful study of some of the many factors governing legume growth, inoculation and nitrogen fixation should lead to more rational ideas and better farming practices.

The indications from the experiments reported in the previous pages may be summarized here briefly in order to direct attention to the more salient points which they bring out.

In the first place, the relative proportion of the total plant growth in the tops and roots of red clover and of alfalfa at different stages of growth and under different conditions should be noted. With red clover on unsterilized Carrington loam, the percentage of total plant growth in the roots varied

from 29 to 34 being the greatest at the period when the blooms appeared. The percentage at maturity was 32. On the same soil sterilized and inoculated, it varied from 27 to 34 per cent, the higher figure again being found when the blooms appeared. At maturity, however, only 27 per cent was in the roots, indicating a variation due to the sterilization of the soil. In the latter case the percentage in the roots decreased from the time when the blooms appeared to maturity, while in the former it decreased to full bloom and then increased.

On the unsterilized Miami fine sandy loam, the percentage varied from 26 to 42, being the greatest at full bloom. There was a gradual increase up to that stage and a slight decrease at maturity. On the same soil sterilized and inoculated, the percentage varied from 32 to 51, being the greatest at the first two stages and gradually decreasing to 32 per cent at maturity.

Conclusions from these figures are very difficult to draw and probably the only safe deduction to be made is that the amount of total plant growth in the roots of red clover varies with the soil, with the inoculation and with the general growth conditions. The variations from 32 to 40 per cent of plant growth in the roots at maturity may indicate the variation which may occur on different soils in the field. The average (36 per cent) may be a fair estimate of the proportion of the red clover plant in the roots at maturity, when grown on unsterilized soil. Under the somewhat artificial conditions brought about by sterilization and inoculation, the average proportion was less, 29 per cent, varying from 27 to 32 on the two soil types. The effect of inoculation, as tested by the method used here, was insignificant, but the sterilization may have changed the effects which inoculation would otherwise occasion. The greatest development of roots of the red clover occurred when the blooms appeared in all but one case, whether or not the soil was sterilized, and in all but one case the smallest proportion of plant growth was in the roots at maturity.

With alfalfa on unsterilized Carrington loam, the proportion of total plant growth in the roots was the greatest at the stage when blooms appeared, 53 per cent, the lowest 2 weeks earlier, 14 per cent, and slightly less at the later periods. On the same soil, sterilized and inoculated the percentages were very similar; 14, at the first period, 44, 46, and 48 at the three later periods, the largest figure being found at maturity. On the uninoculated Miami fine sandy loam, the percentage varied from 24 to 55, being the smallest at the first period and gradually increasing up to maturity. The average percentage for the unsterilized soils, when the alfalfa was at maturity, was 52, and on the sterilized soil, 48; so that the effect of inoculation was apparently not beneficial at least to root development. Whether there was any retardation by sterilization which subsequent inoculation was not able to overcome cannot, of course, be determined from these tests. It is evident, however, that a much larger proportion, about one-half in fact, of the alfalfa growth was in the roots than was the case with red clover which showed 36 per cent. It

is evident also that the period of greatest development of the roots came between the period 2 weeks before the appearance of the blooms and when the blooms appeared, and there is indication that the percentage of total plant growth in the roots, increased as maturity approached.

Considering the nitrogen content of the tops and roots of the red clover and of the alfalfa, there are some deductions which should be drawn from the tests. With red clover on the unsterilized Carrington loam there was very little difference at the different stages of growth in the proportion of total nitrogen of the plant in the roots. It varied from 20 to 24 per cent, and was the smallest at maturity.

On the sterilized soil the results were very similar, varying from 19 to 26 per cent, being again the smallest at maturity and the greatest at the earliest stage of growth. On the unsterilized Miami fine sandy loam, the reverse of these results was secured, the percentage varying from 25 to 34, the greater percentage being found at maturity, and the smallest at the first stage. On this latter soil sterilized and inoculated, the smallest percentage was at maturity, 27, against 51 at the first stage. It seems evident from these results that the percentage of total nitrogen in the roots of red clover depends upon the soil conditions and may be widely different on different soils. When sterilized and inoculated, the results were also very different in the two soils, which might be attributed to the difference in the effect of sterilization. The effect of inoculation was not shown to be of any great significance on the development of root growth or on the increase of the element nitrogen in the roots. In fact there seemed to be a slightly smaller proportion of the total nitrogen in the roots at maturity where the soil was sterilized and inoculated, but this effect may be entirely the result of the sterilization. It may also be due to a greater growth of tops in proportion to roots brought about by the inoculation. As a matter of fact, the plant growth, tops and roots, was increased on the Carrington loam, but the tops were increased to a much greater extent than the roots on the Miami soil; however, the plant growth was less than on the unsterilized soil. This would emphasize the difference in the effect of sterilization on the two soils and indicate that on the Miami soil the sterilization was somewhat injurious to plant growth.

It should also be remembered that the efficiency of the inoculating bacteria may be very different in the two soils. The organisms in the Carrington loam were apparently not as vigorous as the cultures added after sterilization, while in the Miami soil, either the organisms were very efficient in the unsterilized soil or the sterilization proved so injurious that the cultures introduced did not have the best opportunity for development. The latter explanation seems the more tenable, especially in view of the fact that the plant growth itself was restricted.

On the sterilized, inoculated soils there seemed to be more nitrogen in the roots at the earlier stages of growth, which may be an effect of inoculation and of a consequent increase in nitrogen fixed in the roots earlier in the develop-

ment of the plant. At the later stages of growth, the greater development of the tops on the Carrington loam caused a greater proportion of the nitrogen to accumulate above ground. On the Miami soil, as has been noted, the sterilization reduced the plant growth and the percentage of nitrogen in the roots was reduced because of a pronounced reduction in root development. It is quite probable that the inoculation of red clover may make considerable difference in the proportion of total nitrogen present in the roots at different stages of growth. Unfortunately, the sterilization factor complicated the problem here, and no definite conclusions can be drawn. Tests are needed which will show comparative results on uninoculated soils; i.e., soils on which no nodules will be formed, with those obtained on soils abundantly supplied with vigorous bacteria. The inoculation factor is certainly of some importance in determining the relative nitrogen of tops and roots and in showing how much nitrogen will be left in the soil when the clover crop is removed.

It is interesting to note also that there was more nitrogen in the roots of the red clover at maturity on the Miami fine sandy loam than on the Carrington loam. Perhaps there is some relation here between soil type and nitrogen in the roots, the greater content or proportion of this element being found in the roots on soils lower in nitrogen and organic matter. There is another factor to take into account, however, and it will be recalled that the plant growth was much less on the soil poorer in nitrogen. Some relation between total plant growth and nitrogen in the roots may be all that these results should be considered to indicate. It is believed, however, that the type of soil and particularly its content of organic matter and nitrogen will prove an important factor in determining the proportion of nitrogen in the roots of red clover, and these results seem to confirm this idea. There may be exceptions to the indications from these experiments, but they seem rational and serve to call attention at least to the desirability of further tests along this line on widely differing soil types, types whose color and texture are very different.

Considering now the alfalfa, it will be noted that on the unsterilized Carrington loam the proportion of nitrogen in the roots varied from 13 to 44 per cent, being the greatest at maturity and the least at the first stage. Very little difference occurred between full bloom and maturity. When the soil was sterilized and inoculated the figures were very similar, varying from 16 per cent at the first stage to 42 at maturity. Very little difference is shown here between the results on the soil differently handled and treated. In both cases the greatest percentage was found at maturity and the figures were about the same whether or not the soil was uninoculated or sterilized and inoculated artifically. The soil evidently was supplied with very vigorous organisms and the inoculation did not prove of any effect on the root development, or else the sterilization retarded development to just the extent that the inoculation increased it, giving about the same results as on the uninoculted soil. This of course is mere speculation.

On the uninoculated Miami fine sandy loam the proportion of total plant nitrogen in the roots varied from 22 to 47 per cent, being the smallest at the earliest stage and the greatest at maturity; just as was the case on the other soil. The actual proportion of nitrogen in the roots at maturity was greater on this soil than on the Carrington loam which is exactly the same result noted on the clover. The experiment with alfalfa on the Miami soil, sterilized and inoculated, was not carried to completion because of failure of the crop and no comparison can be drawn as to the relative influence of sterilization and inoculation on the two soil types. It seems, however, from the results on the Carrington loam that sterilization and inoculation did not increase the proportion of nitrogen in the roots except at the early stage of growth, which is similar to the results secured with clover.

The amount of nitrogen in the roots of alfalfa at maturity was much greater than in the case of clover, averaging on the two unsterilized soils 46 per cent, while with clover the average was 27 per cent.

Again it seems that on the soil poorer in nitrogen and organic matter, there was a somewhat greater proportion of the total nitrogen in the roots, but the difference was not so pronounced with alfalfa as with clover. Apparently the soil type influenced the proportion of nitrogen in the roots of alfalfa just as was true with clover, and this may be a reflection of the relation between the amount of crop growth of tops and the characteristics, both physical and chemical, of the soil on which the crop is grown.

The amount of nitrogen taken from the air by inoculated red clover and alfalfa plants was considerable, according to the figures obtained in these tests. With red clover on Carrington loam unsterilized, 12.8 cgm. of nitrogen per plant was fixed at full bloom. On the Miami fine sandy loam 15.9 cgm. was fixed per plant at maturity. In both cases the amounts fixed gradually increased from the earlier stages of growth up to maturity. In the first instance the figure obtained at maturity was somewhat less than that at full bloom but no duplicate was secured; so it should not be considered definite. When the soils were sterilized and artificially inoculated the nitrogen fixed per plant at maturity amounted to 17.0 cgm. on the Carrington loam. On the Miami fine sandy loam, however, the fixation was less than on the unsterilized soil, amounting to only 5.9 cgm. per plant. Thus the indications from the results on the proportion of nitrogen in the roots of red clover are borne out by the evidence here that the sterilizing and inoculating of the Carrington loam gave an increase in nitrogen fixed while with the Miami soil the reverse effect occurred undoubtedly due to the sterilization exerting an injurious influence on the Miami soil.

It may also indicate that the bacteria naturally present in the Miami soil were more efficient than those in the Carrington soil, and the introduction of vigorous organisms artificially did not prove of any significance.

There is some difference in the amount of nitrogen fixed by the same legume on different soils, but the results do not permit of definite conclusions here.

They show the greatest fixation at full bloom on the richer soil while at maturity the poorer soil shows more fixation, but in the former case the figures are uncertain, not being duplicated. It seems justifiable, however, to conclude that the soil type bears a very important relation to the nitrogen fixed.

With alfalfa on the unsterilized Carrington loam the fixation of nitrogen increased up to 24.9 cgm. per plant at maturity. On the same soil sterilized and inoculated, the fixation was slightly less, increasing up to 20.3 cgm. per plant at maturity. On the Miami fine sandy loam the fixation increased up to 17.0 cgm. per plant at maturity—a smaller amount than that found on the Carrington loam. The reverse of the test with red clover seems to have occurred here and the greatest fixation on the unsterilized soil occurred on the richer soil. The reverse from the clover results also is true here when the fixation on the sterilized inoculated soil is compared with that on the unsterilized soil. Apparently the inoculation with artificial cultures did not prove of great value. Perhaps the sterilization of the soil proved a deterrent to as great bacterial action as occurred naturally.

Comparing the nitrogen fixed by the two crops it will be noted that the clover fixed less nitrogen than the alfalfa on the Carrington loam, unsterilized, but more than the alfalfa on the Miami soil. This may be explainable on the basis of more efficient alfalfa bacteria in the Carrington loam while the clover bacteria were the more vigorous in the Miami soil. It may also be due to differences in the physical or chemical conditions in the two soils which affect the bacteria from the various legumes differently.

It is apparent from these results that from 12 to 25 cgm. of nitrogen per plant may be secured from the atmosphere by red clover or alfalfa, but this amount, of course, will not all be added to the soil if the tops are all removed and only the roots left in the soil. How much of the total amount of nitrogen taken from the atmosphere by a crop of red clover or alfalfa will be left in the soil if the hay crop is removed? This question is one of considerable significance and the results obtained in these experiments indicate that with red clover on the unsterilized Carrington loam practically all of the nitrogen in the tops came from the air, when the blooms appeared, while at full bloom and later more than that was fixed, some nitrogen apparently being fixed in the roots. On the Miami fine sandy loam almost all the nitrogen of the entire plant came from the air, enough to supply all in the tops and a large part of that in the roots. On the sterilized and inoculated Carrington loam sufficient nitrogen was fixed to just about fill the need of the tops but none extra, while on the Miami soil sterilized and inoculated all the nitrogen in the tops at full bloom came from the air but not quite all at maturity. These results serve to emphasize the fact that sterilization caused some bad effects in the soil and the results secured must be considered abnormal.

With the alfalfa all the nitrogen in the plants at maturity seemed to come from the air but there was evidently some action by the non-symbiotic nitrogen fixers, the azofiers. Apparently, however, all the nitrogen in the tops and a

part of that in the roots came from the air. Similar results were secured on the same soil sterilized and inoculated, and on the Miami fine sandy loam. Unfortunately it was impossible to learn how much nitrogen was fixed by the azofiers but from general information it appears that the amount is insufficient to change materially the results secured here. It may be concluded that with clover and alfalfa on these two soils all the nitrogen in the tops came from the air and a part of that in the roots also was obtained in that way. Even allowing for azofication there may be some increase in nitrogen in the soil from growing inoculated legumes like red clover and alfalfa on soils of high or low nitrogen content, even when the hay crops are removed.

SUMMARY

From these experiments with red clover and alfalfa on Carrington loam and Miami fine sandy loam, the following conclusions may be drawn:

1. On the average, 36 per cent of the total plant growth of clover was in the roots at maturity on the unsterilized soils. On the sterilized soil the percentage was lower.

2. A larger proportion of the total plant growth of clover was in the roots at maturity on the soil poorer in organic matter and nitrogen. On the better soil there was greater total growth and a much greater growth of tops.

3. Over one-half the plant growth (53 per cent) of the alfalfa was in the roots at maturity on the unsterilized Carrington loam—slightly less (48 per cent) on the unsterilized Miami fine sandy loam. On the sterilized, inoculated Carrington, the percentage was slightly lower.

4. The percentage of total nitrogen in the roots of clover and alfalfa was greater on the soil poorer in organic matter and nitrogen.

5. On the average 27 per cent of the total plant nitrogen was in the roots of clover at maturity under natural soil conditions, while with alfalfa an average of 46 per cent of the total plant nitrogen was in the roots.

6. With clover there was a greater fixation of nitrogen on the poorer soil while with alfalfa the greatest fixation was on the better soil.

7. From 12 to 25 cgm. of nitrogen was fixed per plant by clover and alfalfa on untreated soils.

8. With clover on both soils unsterilized all the nitrogen in the tops and some of that in the roots came from the air. On the Miami soil a larger proportion of the nitrogen in the roots came from the air than on the Carrington.

9. With alfalfa all the nitrogen in the tops and some in the roots came from the air.

10. When clover and alfalfa are grown and the hay crops removed, there may be some gain in nitrogen in the soil, the amount of the increase of the element in the soil varying with the legume, the soil type, the inoculation and the general growth conditions.

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PRELIMINARY NOTE ON THE MICROBIOLOGY OF THE SOIL AND THE POSSIBLE EXISTENCE THEREIN OF INVISIBLE GERMS

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Everyone is familiar with the fact that very little is known about the bacterial flora of the soil (bacteria, yeasts and fungi) not only from the viewpoint of tabulation of the species existing therein, but especially from the viewpoint of its significance.

Accurate studies of soil bacteriology, moreover, are lacking for a number of reasons, as will appear later, not the least important of which is the fact that for a long time the hygienists have occupied the field in this matter, especially considering the presence of bacteria in the soil as a contamination. This has led particularly to the study of soil bacteria as a manifestation of the injurious action of pathogenic species.

At present, however, it is well understood that the microbic flora forms an essential element of the fertility of an agricultural soil and that the pathogenic germs *par excellence* of the soil, those of tetanus and malignant oedema, are probably only paratrophic forms of quite common meta and prototrophic germs, viz., those of the group of butyric acid bacteria closely related to the amylobacteria, all of these being forms that must have, even if we do not yet know its character or extent, an important share in that great process, the decomposition of the cellulose under the surface of the soil.

Now the other reasons may be reduced perhaps to a single one, viz., the vast uncertainty that surrounds everything that has to do with the fundamental methods of research. Therefore, he who wishes to make a serious study of the soil from the microbiological viewpoint must first serve an apprenticeship, not only in order to master the methods, but also in order to form a personal opinion of these, which frequently extends to a criticism of these methods and even to their rejection, or at least to a loss of confidence in them. And to indicate merely a few phases of the problem affected by these deficiencies it will be sufficient to mention:

A. *The uncertainties in the computation of the microbes in the soil*, because of which through a slight change of method, there is an enormous change in numerical result.

B. *The difficulties proceeding from the detrimental influence that the number and variety of microorganisms are capable, during development, of exerting upon each other.* This we have noted several times and particularly as a basis for a general

statement which we have made and which may be expressed thus: In the majority of cases the number of germs that can be successfully developed from the soil, by isolating cultures diluted with agar-agar and gelatine, is inversely proportional to the quantity of soil infusion employed for the determination. Thus, for instance, with 1/15 cc. of soil infusion there is generally obtained a larger number of germs than with 0.1 cc. One of a series of experiments, for instance, contains these figures:

a. In 23 analyses, 23 cases gave a greater development of germs from 0.02 cc. than from 0.1 cc.

b. In 23 analyses, 22 cases gave a greater development of germs from 0.04 cc. than from 0.1 cc.

c. In 34 analyses, one-half of the cases (17) gave a larger number of germs which developed from 0.04 cc. and in another half of the cases (17) a larger number was obtained with a 0.1 cc. sample.

C. *The influence of the methods employed for the isolation of the microbial species of the soil*. At present, it is clearly proven from the experience we have had for so many years that the examination of the soil for microbial species depends upon the method of isolation employed and especially upon the nutritive character of the medium selected. Agar-agar or gelatine (or even potatoes), however prepared, never gives anything different from the usual mesentericus, fluorescens, pigmented cocci, radiobacter (radiciformi ?), streptotriceae, molds, etc., and there are always in the soil, a few germs such as the *B. mycoides* which, if there are but a few individuals, are sufficient to hinder the development of all the other species.

And what should be done in case of all the other groups? For these we must always have recourse to selected enriched cultures which indicate absolutely nothing as to the actual relative number of germs, and hence as to the importance of these groups in the soil, not to say that even these selected culture methods are very frequently inadequate, hence the following difficulty:

D. *The inadequacy of the methods as applied to the isolation of the given species.* We have for instance devoted much time to isolation of the nitrifying bacteria and have convinced ourselves that quite correctly Winogradsky, Migula and Warington have regarded their presence as entirely salutary. Frequently on infecting with soil even eight to ten large flasks of selected nutritive liquids we have in none developed nitrite or nitrate bacilli.

E. *The enormous difficulty of sterilizing the soil completely*. Everybody is aware that the autoclave and the steam sterilizer of Koch, within normal time limits, are not successful in sterilizing the soil; neither is the hot-air sterilizer, nor roasting by direct heat.

This especially has been the reason why for some time we have been proceding cautiously in experimenting on the soil. It is indeed a standing rule of this institute to hold that no importance can be assigned to any microbe whatsoever in any medium whatsoever if we cannot repeat the phenomenon with the microbe in question, with a pure culture, and not only in the particular

medium of its own natural flora, but also without altering this meduim in its character and properties. We should consider that the soil is a complex chemico-physical structure. It contains colloids coagulable by heat. Moreover, it contains a circulating solution that may be considered *the true medium* of the soil in which microbes act, producing reactions and sustaining their influence.

I mean that since microbes form the *biological basis* of the complex system, it is clear that any sterilization, chemical or physical, must affect profoundly the capacities of a method so liable to falsify the results of any possible experiments.

F. *The lack of sensitiveness of certain chemical methods*, which, on the other hand, are the only ones at our command in order to understand the reactions produced by microbes, is also an obstacle. Of this we need give only two examples, viz., the lack of sensitiveness of methods for the determination of nitrogen which is necessary for the study of nitrogen-fixing organisms, and for which there has been found thus far nothing better than the fundamental method of Kjeldahl and its modifications, revealing only quantities that are calculable in a few milligrams; and the difficulty of the search for nitric acid in the presence of large quantities of nitrous acid, for which, even qualitatively, we possess nothing more sensitive than the very inadequate method of Lunge-Luvoff.

Wherefore, to sum up, we believe that we must be very cautious about regarding as fully known the *entire* micro-biological mechanism of certain soil phenomena such as those of nitrification and the fixation of nitrogen. It is insufficient for their interpretation to have succeeded in isolating from the soil germs which when placed in glass nitrify or fix nitrogen. It would be as if we should admit that in the soil there is alcoholic fermentation as an essential phenomenon simply because we can isolate from the soil yeasts which produce this process.

Hence all this obscures very much all that we know, not indeed concerning the biochemical action of many groups of germs, but certainly concerning their *real value* in the soil.

Furthermore, it is important not to forget that many of the theories concerning the soil processes are based upon phenomena which occur and are studied with germs isolated from the soil and allowed to act in artificial solutions which can certainly not be compared with the circulating solution.

It is therefore the inadequacy of the recognizable microbic flora in the soil to explain many biochemical phenomena that here occur, which has led us to seek the possible presence of an invisible, or rather an ultramicroscopic flora, or indeed to solve the question whether there exist invisible germs in the soil.

A direct method has been employed, that is, it consisted of taking fresh soil, mixing it with ordinary water in equal weights and allowing it to decant for 30 minutes, filtering the decanted liquid with the porous candles by means of the Gay-Lussac pump in the Chamberland apparatus. The liquid containing the supposed invisible germs would descend aseptically into Erlenmeyer flasks

containing culture liquids aseptic by test, in which the aforesaid microorganisms might produce the anticipated reactions.

About 50 cc. were filtered off which were mixed with 300 cc. of test liquid, and the 50 cc. were measured by a mark previously made with a diamond on the Erlenmeyer flasks.

It was the writer's intention to observe the principal reactions that are attributed to invisible germs, viz., putrefactive reactions (test for ammonia, indol and phenol), nitrite and nitrate reactions, and fixation of nitrogen. Naturally it was necessary to ascertain beforehand that the filtered liquid did not already contain the principles which it should produce.

From the researches made thus far with the soil of Gusserie Park of our own school (in the months of April and May) we have been able to deduce that:

1. The filtered infusion of soil (which we might also call circulating solution) does not contain indol but does contain ammonia and nitrites.

2. The results have been negative in an attempt to produce indol (tested with the familiar nitroso-indol reaction) from the nutritive liquid containing peptone. Needless to say, the same liquid unfiltered produced it abundantly as a result of the ordinary agents of putrefaction.

3. The sterile circulating solution did not cause any increase in nitrites beyond those which were present naturally in a liquid of the following composition:

Distilled water	100.00
Ammonium sulfate	0.01
Dipotassium phosphate	0.10
Magnesium sulfate	0.05
Sodium chloride	0.20
Ferrous sulfate	0.04

After sterilization, an excess of magnesium carbonate was added.

This last experiment was conducted in the following manner. To two Erlenmeyer flasks with 300 cc. of the aforesaid sterilized liquid was added 50 cc. of the sterile circulating solution. One of the two was then sterilized to serve as a control and the other preserved in a thermostat at 28° to 30°C. Two weeks later the contents were tested in nitrites and were found unchanged by the Gries reaction.

Clearly this is insufficient to disprove invisible germs. The experiments are still being conducted and surely will be interesting whatever their outcome.

THE COLORIMETRIC DETERMINATION OF SOIL IN A COLORED WATER EXTRACT

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Recently it became necessary, in connection with other investigations, to make a large number of nitrate determinations on soils containing varying and often excessive amounts of actively decomposing organic matter. The filtrates from the water extract of these soils were colored a deep brown, resembling a weak caramel solution. This color persisted despite attempts to remove it by additions of the usual decolorizing agencies. As it was necessary to make the determinations as rapidly as possible and because it has been shown (5) that the phenoldisulfonic acid method is extremely accurate in the absence of chlorides and sulfates (neither of which were present in sufficient amount to interfere), means were sought to decolorize the solutions in order that the nitrates might be determined colorimetrically.

Greaves and Hirst (6) in discussing the preparation of a clear filtrate state, "it would appear that the most likely flocculants are sodium, potassium and iron alum; ferric sulfate; lime; finely precipitated calcium carbonate and talc." They were working with soils containing alkalis in varying amounts and only normal amounts of organic matter. They found that their solutions could be clarified by the addition of 2 gm. of alum to the soil by filtering through a Pasteur-Chamberland filter or by centrifuging.

These flocculants, however, failed to decolorize the soil solutions obtained in this work despite the fact that the quantity added to the soils varied from 1 to 10 gm. Filtrations were made through Pasteur-Chamberland filters of medium fineness, through 3-inch alundum cones and through Büchner funnels using Whatman no. 31 discs of double thickness. As no trouble was experienced in obtaining a clear but highly colored solution the centrifuge was not used. Other agents—lamp black, bone black, powdered charcoal, animal charcoal, charcoal from blood and a commercial organic carbon Norit[2] reported by Bradley (3) were tried, but with the single exception of the latter all failed to decolorize completely. The Norit, however, was objectionable in that it contained a measurable amount of nitrates and the investigation was continued in the hope of securing an agent common to all laboratories.

[1] Contribution from Department of Farm Crops and Soils, Iowa State College, W. H. Stevenson, Professor in charge.

[2] Sample furnished by the Joseph Baker Sons & Perkins Company, Incorporated, White Plains, New York.

Attempts were then made to oxidize the organic matter by the use of potassium permanganate as described by Syne (7). After a thorough trial this method was discarded on account of a development of an interfering color in the subsequent evaporation to dryness. Bromine was then substituted for permanganate as an oxidizing agent and a colorless solution secured that dried in a satisfactory manner. The results, however, showed too low a nitrate content. When known amounts of nitrates were added to the colored solution before oxidizing it was impossible to recover over 25 per cent.

The next method tried was the aluminum reduction method of Burgess (4) as described in the official methods(2). These methods make no provision for soil nitrate determinations in a colored extract but state that the nitrates must be reduced to ammonia by aluminum in case more than 6 parts per million of chlorine are present. The inference is conveyed that in case of failure to secure a clear solution the reduction method is to be used. This method, aside from the time required for the determinations, was found to be objectionable on account of three exceedingly variable factors; namely, time for complete reduction, temperature at which reduction takes place, and nitrogen impurities in the aluminum foil.

A further search of literature revealed the fact that aluminum hydrate is recommended as a clarifying agent in water analysis (1). This may be prepared either by electrolizing ammonia-free water with the use of aluminum electrodes, or by precipitating the hydrate from an alum solution with ammonium hydroxide. In either case the precipitate must be washed by decantation until free of chlorine, ammonia and nitrates. This agent was found to remove all the color from the soil and manurial extracts used in this work, whether they were obtained from fresh or dried samples. As a result, attempts were made to perfect a method with this agent, using, as a basis of comparison, Norit as a decolorizing agent and the aluminum reduction method without decolorizing.

A large amount of aluminum hydrate was prepared from potassium alum; washed free of ammonia, nitrates and chlorides; allowed to concentrate as much as possible, and, after the volume of the concentrate had been determined, stored in a glass bottle for further use. At first definite amounts of the aluminum hydroxide solution were added directly to the soil solution before shaking. This procedure made the subsequent filtration difficult, as aluminum hydrate is primarily a decolorizing agent. The pores of the Pasteur-Chamberland filter were absolutely clogged. Better results were secured when 5 gm of precipitated calcium carbonate were used in addition. A decided improvement was made by adding 5 gm. of precipitated calcium carbonate directly to 100 gm. of soil, adding 400 cc. of water, shaking 15 minutes and filtering through a Pasteur-Chamberland filter. The color was then removed by adding a sufficient amount of aluminum hydrate filtering through a coarse filter paper and thoroughly washing the filtrate, directing the stream in such a manner as to free the gelatinous mass from the paper. The solution was then evaporated to dryness and the nitrates determined colorimtrically with phenoldisulfonic acid in the usual manner.

It was noticed that varying amounts of the agent were required to decolorize solutions of varying density. In searching for some material to give an indication of the proper amount to add it was found that a weak solution of caramel $C_{12}H_{18}O_9$ gave a color concentration very similar to that of the soil and equivalent amounts of hydrate would decolorize either. Accordingly, a 1 per cent caramel solution was prepared by dissolving 1 gm. in 100 cc. of water. A series of standards was then made by using 1, 2, 3, etc. cc. of this stock solution

TABLE 1

*Nitrate nitrogen recovered from colored soil solution variously treated**

SOURCE OF SAMPLE	COLOR OF SOIL SOLUTION IN TERMS OF STANDARD	NUMBER OF DETERMINATIONS IN AVERAGE	SOLUTIONS DECOLORIZED NITRATES DETERMINED BY PHENOLSULFONIC METHOD								SOLUTIONS NOT DECOLORIZED
			Decolorized with aluminum hydrate				Decolorized with norit				Aluminum reduction method
			Nitrates in soil	Nitrates added	Total nitrates recovered	Proportion of added nitrates recovered	Nitrates in soil	Nitrates added	Total nitrates recovered	Proportion of added nitrates recovered	Nitrates in soil
			mgm.	*mgm.*	*mgm.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	*per cent*	*mgm.*
Soil from under manure pile..	9	4	25.51	10.00	35.21	97.0	26.42	10.00	30.30	38.8	24.23
Soil plus 36 tons of decomposing manure......	2	4	2.05	10.00	11.85	98.0	2.00	10.00	2.69	6.9	2.37
Normal loam soil..........	Clear	4	1.51	10.00	11.56	100.5	1.96	10.00	11.44	94.8	1.74
Normal loam soil not treated with decolorizing agents.......	Clear	2	1.48	10.00	11.03	95.5					

* Results calculated on basis of 100 gm. of air-dry soil.

and building up to 100 cc. with distilled water. A scale ranging in concentration from 1 cc. of stock solution to 5 cc. will usually be sufficient for most soils, but manurial extracts will give a color concentration equivalent to a 15 or 20 cc. concentration. In this work it has been found that an aluminum hydrate solution containing the equivalent of $\frac{3}{4}$ gm. of potash alum is sufficient to decolorize an extract having a color concentration equal to (1) or 1 cc. caramel stock solution. Other concentrations will require 2 or 3, etc. times as much hydrate in regular rotation.

Results secured by the above method are submitted in table 1. These results were selected from a number of determinations made to test the efficiency of aluminum hydrate in decolorizing the soil solution for a determination of nitrates by the phenoldisulfonic acid method. The efficiency of Norit also is compared, while both are compared with the aluminum reduction method.

There is no question regarding the efficiency of either aluminum hydrate or Norit in successfully clarifying a soil solution without loss of nitrates. If, however, nitrates are added to the solution before decolorizing they are readily washed free from the aluminum hydrate precipitate, but are apparently partially absorbed by Norit. Since these resolutions were secured it has been found that the method may be modified to the extent that only one filtration is necessary. Five grams of precipitated calcium carbonate and 400 cc. of distilled water are added to 100 gm. of air- dry soil in 1-liter bottles. The soil is shaken 15 minutes, then allowed to settle 30 minutes. A 100-cc. pipette is then connected to suction pump with a long piece of tubing and an aliquot is quickly drawn off. A rubber stopper placed on the delivery tube of the pipette at such a height that the upper level of the solution only will be drawn off is of great assistance. The proper amount of aluminum hydrate is added to the aliquot, filtered through a coarse grade of paper and thoroughly washed. In this way the removal of color is a very rapid procedure.

SUMMARY

Soil solutions containing large amounts of soluble organic matter may be quickly decolorized by aluminum hydrate and the nitrate-nitrogen content determined colorimetrically by the phenoldisulfonic acid method.

The amount of aluminum hydrate necessary to decolorize the solution is quickly determined by comparison with a standard.

The method is accurate and exceedingly rapid. One filtration through a coarse quick filter paper removes all color.

A flocculating agent must be used to secure rapid results on fine-grained soils. Precipitated calcium carbonate is recommended.

No chemical reaction takes place that later may influence the development of color.

The decolorizing action is immediate.

Aluminum hydrate is prepared by dissolving 125 gm. of potash or ammonia alum in 1 liter of water. Add cautiously sufficient ammonium hydroxide to turn red litmus blue. Wash by decantation until free of ammonia.

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THE USE OF SILICA CRUCIBLES FOR THE DETERMINATION OF POTASSIUM IN SOILS

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Fusion of the finely ground material with a mixture of ammonium chloride and calcium carbonate in a platinum crucible of special design is perhaps the most distinctive feature of the J. Lawrence Smith method for the determination of potassium in rocks, minerals and soils. The shape and length of the crucible are such as to enable one to heat strongly the lower portion containing the charge while keeping relatively cool the upper portion to prevent loss of potassium through volatilization of its chloride. In the use of the J. Lawrence Smith crucible it is customary to insert it in an inclined position to any desired depth through the wall of a clay or asbestos board cylinder. The clay or asbestos board is sufficient protection against overheating the upper portion. This combination is entirely satisfactory only when one has good gas and comparatively few determinations to make.

In this state a large number of potash determinations are involved each year in connection with soil survey work. The enormous increase in the price of platinum made impracticable even several years ago the addition of new platinum crucibles to facilitate the analytical work. Our first substitutions were nickel crucibles of the J. Lawrence Smith type. Their first cost was much less. The life of the nickel crucible in this work, however, is comparatively short. Moreover, heating the crucibles in the manner recommended becomes somewhat tedious and cumbersome when the analytical work incident to the soil survey is reduced to a routine and energetically pushed. These reasons, together with our desire to reduce to a minimum the use of an unsatisfactory gas supply, induced us to try out for this work another substitute for platinum and finally to adopt a somewhat different procedure from what has been recommended heretofore for the fusions. The experimental work which follows justifies the modified procedure.

EXPERIMENTAL

The cracking of a nickel crucible of the J. Lawrence Smith type during a fusion and our inability to replace it at once led to the suggestion that several fusions be made in a silica crucible of the shape generally used for ignitions for comparison with fusions made at the same time of the same soils in a J. Lawrence Smith platinum crucible. We were somewhat curious to know how the

crucible would stand up under the treatment. The first fusion, made by direct heating with gas, was slaked at once in the usual manner and its washed insoluble residue digested with dilute hydrochloric acid. The fusion had been perfect. The potash content of this fusion was then determined, as was that of two subsequent fusions of other soils. The same crucible, a no. C1, vitreosil, porcelain shape—glazed, was used for the three fusions. A ½-gm. sample of soil was used in each fusion. The weights of potassium platinic chloride and corresponding percentages of potash obtained from fusions in the silica crucible and in the J. Lawrence Smith platinum crucible are stated in table 1.

After slaking there appeared to be a slight residue on the bottom of the silica crucible which required scraping for its complete removal. To find out whether

TABLE 1

Potash in soils by fusion in a platinum crucible of the J. Lawrence Smith type, and in a silica crucible of porcelain crucible shape

LABORATORY NO.	SOIL TYPE	FUSION IN J. L. S. PLATINUM CRUCIBLE		FUSION IN SILICA CRUCIBLE	
		K_2PtCl_6	K_2O	K_2PtCl_6	K_2O
		gm.	*per cent*	*gm.*	*per cent*
11435	Clay loam	0.0468	1.81	0.0470	1.82
11420	Wheatland silt loam	0.0333	1.29	0.0341	1.33
11408	Wapato silt loam	0.0397	1.54	0.0385	1.50

TABLE 2

Potash in soils by fusion of 1-gm. samples in silica and platinum crucibles of ordinary shape for 50- and 70-minute periods in a gas-fired muffle furnace

LABORATORY NO.	SOIL TYPE	40-MINUTE FUSIONS (P) PLATINUM (S) SILICA		70-MINUTE FUSIONS (P) PLATINUM (S) SILICA		FUSION IN J. L. S. PLATINUM CRUCIBLE	
		K_2PtCl_6	K_2O	K_2PtCl_6	K_2O	K_2PtCl_6	K_2O
		gm.	*per cent*	*gm.*	*per cent*	*gm.*	*per cent*
11407	Salem loam	0.1005 (P)	1.95	0.0995 (S)	1.94	0.1008	1.95
11429	Cascade loam	0.1054 (S)	2.04	0.1062 (P)	2.06	0.1066	2.06

this slight residue retained an appreciable amount of potash, fusions were next made in a gas-heated muffle furnace of 1-gm. samples of two other soil types—two in silica crucibles (no. C1) and two in platinum crucibles of the ordinary shape used for ignitions. The muffle was heated to dull redness previous to the insertion of the charged crucibles and was not permitted to get a great deal hotter at any time during the fusions. At the end of 40 minutes, one silica and one platinum crucible were removed. The remaining ones were removed at the end of 70 minutes. All fusions were then slaked and run for potash in the usual manner. The results are shown in table 2.

Upon examination of the crucibles after slaking, washing and drying, the platinum crucibles were found to be perfectly clean, and the silica ones to have

a slight residue adhering very tightly to the surface of the bottoms. Since potash in fusions made in the platinum crucibles checked a little more closely with the potash in fusions from the J. Lawrence Smith crucible than did potash from fusions in the silica crucibles, it would seem that a trace at least of potash was held back by the residues not readily slaked and removed by water. The possibility of losing potash by volatilization from the open crucibles was not overlooked, but apparently in the several fusions thus far made that loss was practically negligible. The closeness with which potash, in fusions thus far made in crucibles of the ordinary shape used for ignitions, checked with potash from fusions made in the J. Lawrence Smith platinum crucible, was remarkable. The silica crucible, in which four fusions had been made, appeared to be none the worse for the treatment received. A careful review of the situation brought us to the conclusion that although our experimental data at the time would support the proposal to substitute silica crucibles of simple design for the far more expensive J. Lawrence Smith platinum crucibles in soil analysis, the conditions under which they might satisfactorily and safely be used ought to be established. The advantages to be gained in time and labor by being able to make from eight to twelve fusions at once were sufficient inducements to carry the work farther than we originally intended. We determined to try out the adaptability of the electric muffle furnace for this work.

POTASH FROM FUSIONS MADE UNDER CONTROLLED CONDITIONS

It is evident that, on the one hand, the temperature of the crucibles must be sufficiently high to insure perfect fusion of their contents, and, on the other, that they should be kept below the temperature at which volatilization of potassium chloride is appreciable. Unless otherwise stated all fusions in silica crucibles mentioned hereafter were made in an electric muffle furnace equipped with a rheostat for temperature control. All fusions in the nickel and platinum crucibles of J. Lawrence Smith pattern were made with gas in the usual manner. Results from fusions in the platinum crucible of this type were accepted as the standard by which to judge all other results.

The furnace used is of Hoskins manufacture. It takes a current of 18.8 amperes at 110 volts. The first fusions were made without the use of a temperature indicator of any kind. The fusions were good but the results expressed in percentages of potash were much too low. It was evident that we had greatly exceeded the temperature at which volatilization of potassium chloride takes place. The muffle, 13 by 5 by 4 inches, has a circular opening in the back through which a thermo-couple was inserted when subsequent fusions were made so that its hot junction was about 4 inches from the front of the muffle and about $\frac{3}{8}$ inch above its floor. Readings were taken on a millivoltmeter and corresponding degrees of temperature from its calibration curve.

It was recognized that two factors, temperature and time, must be considered in making the fusions. It was impossible to eliminate completely the time element in determining the maximum temperature at which the crucibles must

be removed to avoid loss of potassium by volatilization. But, by so setting the rheostat arm as to permit of the maximum temperature aimed at being reached in the minimum of time, the influence of the time element was reduced to the minimum.

Temperature limits for fusions

In the first series, five charges of Willamette silt loam from the College Farm in duplicates of 0.5 gm. each in no. C1 silica crucibles, were placed in the muffle at room temperature. The current was then turned on. When the reading on the millivoltmeter reached 28, two crucibles were removed. As the reading on the millivoltmeter increased, two crucibles were removed for each increment of temperature corresponding to two points. The maximum reading was 36. The several fusions were then slaked and potash determined in the usual manner.

TABLE 3

Effect of muffle temperature on potash determinations

A. WILLAMETTE SILT LOAM BY FUSION IN J. L. S. PLATINUM CRUCIBLE, ½ GM., K_2PtCl_6, 0.0781 GM., 3.03 PER CENT K_2O				B. NO. 11707, KIRBY GRAVELLY LOAM BY FUSION IN J. L. S. PLATINUM CRUCIBLE, 1 GM., K_2PtCl_6, 0.0762 GM., 1.48 PER CENT K_2O		
Reading on milli-voltmeter	Time from turning on current	K_2PtCl_6	K_2O	Time from turning on current	K_2PtCl_6	K_2O
	minutes	*gm.*	*per cent*	*minutes*	*gm.*	*per cent*
28	80	0.0541	2.10	66	0.0490	0.95
		0.0567	2.20		0.0464	0.90
30	100	0.0767	2.97	77	0.0748	1.45
		0.0760	2.95		0.0746	1.44
32	117	0.0778	3.02	88	0.0770	1.49
		0.0768	2.98		0.0750	1.47
34	130	0.0715	2.77	104	0.0732	1.42
		0.0708	2.75		0.0722	1.40
36	160	0.0683	2.65			
		0.0664	2.57			

In the second series, four charges of soil no. 11707, Kirby gravelly loam, in duplicates of 1 gm. each, were treated in precisely the same manner as were the five charges of the first series. The maximum reading reached on the millivoltmeter, however, was two points lower. In this series of fusions the potash also was determined by the usual methods of procedure after slaking in water. Results for the two series are expressed in table 3 for comparison with results from fusions of the same soils in the J. Lawrence Smith platinum crucible. Figure 1 presents the same results graphically.

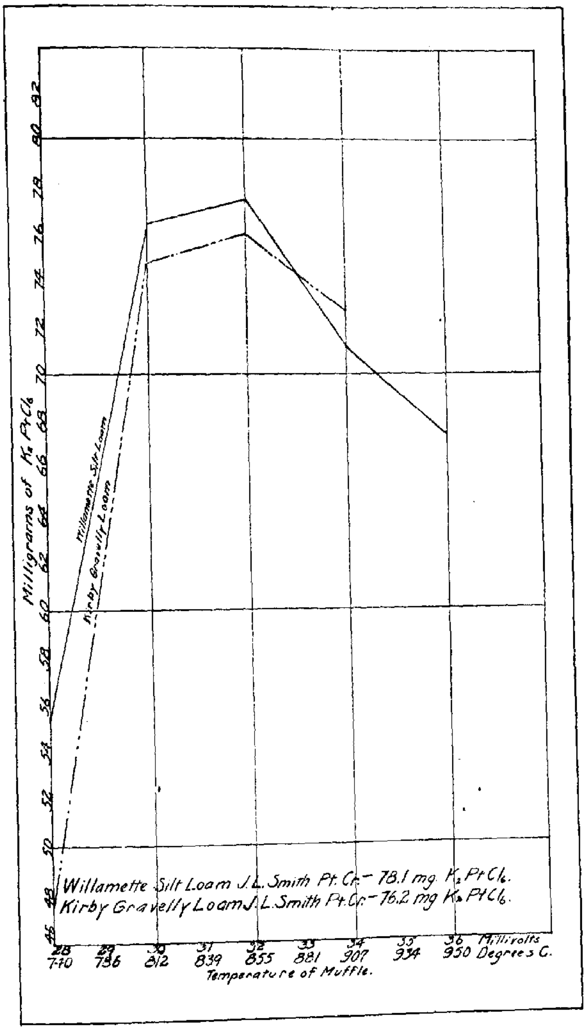

FIG. 1. EFFECT OF MUFFLE TEMPERATURE ON LOSS OF POTASH BY VOLATILIZATION

Fusions below reading 30 were noticeably imperfect; those above reading 30 were perfect, as one would judge them by examination of the residues insoluble in hydrochloric acid. Percentages of potash from fusions below reading 30 and above reading 32 were decidedly low. We concluded: (a) That to secure perfect fusions, in a reasonable time at least, the temperature corresponding to reading 30 on the millivoltmeter, 812°C., must be reached; and (b) that reading 32, corresponding to 855°C., should be the maximum aimed at, or, if the rheostat arm is set for a higher temperature, as perhaps it should be, the crucibles should be removed promptly when that reading is reached to avoid appreciable loss of potassium chloride by volatilization.

The time element

While the conclusions reached above are warranted from the data on the two soils used, it is conceivable that less danger of loss of potassium might be incurred and perfect fusions still obtained by fusing for a longer time at a lower temperature. We next determined how long the charges might be safely held at this lower temperature, 812°C.

TABLE 4

Effect of time of fusion on potash determinations; temperature constant at 30 millivolts, 812°C.; Willamette Silt loam, 0.5 gm.

TIME OF FUSION	K_2PtCl_6		K_2O	BY FUSION IN J. L. S. PLATINUM CRUCIBLE	
				K_2PtCl_6	K_2O
minutes	*gm.*	*gm.*	*per cent*	*gm.*	*per cent*
60	0.0774	0.0760	2.99 2.95	0.0781	3.03
90	0.0767	0.0779	2.97 3.02		
120	0.0771	0.0781	2.99 3.03		
150	0.0747	0.0748	2.89 2.90		

The rheostat arm of the furnace was so set that the maximum temperature aimed at corresponded to reading 30 on the millivoltmeter. When that temperature was reached, four crucibles in duplicate, each charged with 0.5 gm. of Willamette silt loam, were placed in the muffle. The drop of 1½ to 2 points was quickly recovered and reading 30 maintained for 150 minutes. Beginning with 60, two crucibles were removed at the end of each 30 minutes thereafter. The fusions were then all slaked and their potash content determined with the results stated in table 4 and expressed graphically by figure 2.

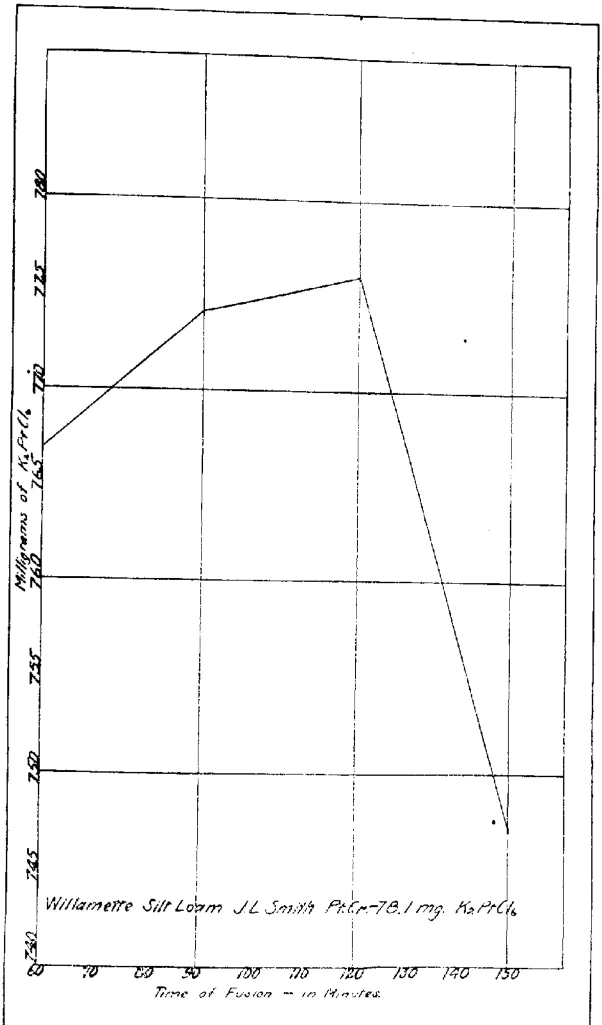

FIG. 2. INFLUENCE OF TIME OF FUSION ON LOSS OF POTASH BY VOLATILIZATION

It would seem that a fusion of at least 60 minutes at this lower temperature (812°C.) is necessary and that heating for another hour is not only safe but advisable to secure perfect fusions. Heating at 812°C. for more than 2 hours results in the loss of appreciable amounts of potassium by volatilization.

APPLICATION OF THE MODIFIED PROCEDURE TO ROUTINE ANALYSIS OF SOILS

Having established the temperature limits around which fusions of at least two types of soil in silica crucibles of simple pattern could be satisfactorily made, we selected a series of samples representing 12 soil types, surface and subsurface, as they occur in Josephine County for the determination of their potash content. Additional fusions of the 12 surface soils were made in both nickel and platinum crucibles of the J. Lawrence Smith type, but of the subsurface soils additional fusions were made in the J. Lawrence Smith platinum crucible only. Results from the fusions in silica crucibles were considered low or high as they varied from results secured from fusions made in platinum. One-half-gram samples were used in all cases. The procedure first outlined for the silica crucible was followed, inasmuch as there would be an appreciable saving of time if that procedure should prove to be generally applicable.

The charged silica crucibles, four in duplicate, were placed in the cold muffle with the rheostat arm of the furnace so set as to allow the maximum reading desired on the millivoltmeter being reached in the mimimum time. Approximately 1 hour and 20 minutes was required to reach reading 32. Promptly on reaching that reading the crucibles were removed from the muffle. The fusions were then cooled and leached and their potash content determined. Results for the 12 surface and corresponding subsurface soils, no two of the same type, are recorded in table 5.

A critical examination of table 5 will develop the fact that the percentages of potash of the 12 surface soils determined in the fusions in the three kinds of crucibles are in close agreement, with two exceptions. No. 11702 and 11705 fusions in silica do not check as closely as good work demands with the fusions in nickel and platinum. Since digestion with hydrochloric acid of the residues insoluble in water did not reveal unattacked mineral, we concluded, after rerunning them in precisely the same manner with practically the same results, that for these two soils the temperature of the muffle had been too high. For similar reasons the same conclusion was reached with reference to no. 11679 among the subsurface samples. These three soils were therefore rerun for potash by fusion for 2 hours with the reading on the millivoltmeter held at 30—corresponding muffle temperature 812°C.—with the results indicated also in table 5.

Judged by results secured with the surface soils alone, the tendency would seem to be for fusions in silica crucibles to run a trifle low in comparison with fusions made in the J. Lawrence Smith platinum crucibles. Judged by results secured on the subsurface soils alone the fusions in silica crucibles would seem to be a trifle high. It is possible that this difference between fusions of surface and

TABLE 5

Potash in soils by fusion of 0.5-gm. samples in silica crucibles with the electric muffle furnace

LABORATORY NO.	SOIL TYPE	FUSIONS IN SILICA CRUCIBLES		FUSIONS IN J. L. S. NICKEL CRUCIBLES		FUSIONS IN J. L. S. PLATINUM CRUCIBLES		DIFFERENCE BETWEEN FUSIONS IN SILICA AND IN PLATINUM
		K_2PtCl_6	K_2O	K_2PtCl_6	K_2O	K_2PtCl_6	K_2O	
	A. Surface soils							
		gm.	*per cent*	*gm.*	*per cent*	*gm.*	*per cent*	*per cent*
11701	Colman gravelly sandy loam	0.0369 0.0366	1.43 1.42			0.0364 0.0366	1.41 1.42	+0.015
11702	Barron coarse sandy loam	0.0562 0.0569	2.18 2.21	0.0585	2.27	0.0598	2.30	−0.105
11702*	Barron coarse sandy loam	0.0598 0.0598	2.30 2.30					0.000
11703	Esterly gravelly clay loam	0.0368 0.0368	1.43 1.43	0.0368	1.43	0.0370	1.44	−0.010
11704	Columbia sandy loam	0.0348 0.0339	1.35 1.32	0.0329	1.28	0.0348	1.35	−0.015
11705	Aiken clay loam	0.0183 0.0164	0.71 0.64	0.0204	0.79	0.0203	0.79	−0.115
11705*	Aiken clay loam	0.0199 0.0192	0.77 0.74					−0.035
11706	Grants Pass clay loam	0.0180 0.0160	0.69 0.62	0.0176	0.68	0.0176	0.68	−0.025
11707	Kirby gravelly loam	0.0371 0.0375	1.44 1.46	0.0391	1.51	0.0381	1.48	−0.030
11708	Esterly gravelly loam	0.0306 0.0315	1.19 1.22	0.0314	1.22	0.0317	1.23	−0.025
11709	Kirby loam	0.0260 0.0253	1.01 0.98	0.0267	1.03	0.0263	1.01	−0.015
11710	Tokay clay loam	0.0170 0.0182	0.67 0.70	0.0174	0.68	0.0174	0.68	+0.005
11711	Holland coarse sandy loam	0.0561 0.0567	2.18 2.20	0.0582	2.25	0.0575	2.22	−0.030
11712	Colman loam	0.0513 0.0524	1.99 2.20	0.0506	1.97	0.0514	1.99	+0.015

TABLE 5—*Continued*

Potash in subsurface soils by fusion of 0.5-gm. samples in silica crucibles with the electric muffle furnace

LABORATORY NO.	SOIL TYPE	FUSIONS IN SILICA CRUCIBLES		FUSIONS IN J. L. S. PLATINUM CRUCIBLES		DIFFERENCE IN AVERAGES
		K_2PtCl_6	K_2O	K_2PtCl_6	K_2O	
	B. Subsurface soils					
		gm.	*per cent*	*gm.*	*per cent*	*per cent*
11641	Colman gravelly sandy loam	0.0333	1.29	0.0324	1.26	
		0.0336	1.30	0.0321	1.25	+0.040
11643	Barron coarse sandy loam	0.0586	2.27	0.0576	2.23	
		0.0568	2.21	0.0573	2.22	+0.015
11646	Esterly gravelly clay loam	0.0325	1.26	0.0319	1.24	
		0.0325	1.26	0.0315	1.22	+0.030
11653	Columbia sandy loam	0.0274	1.06	0.0268	1.04	
		0.0270	1.05	0.0279	1.08	−0.005
11638	Aiken clay loam	0.0102	0.39	0.0094	0.36	
		0.0096	0.37	0.0093	0.36	+0.020
11673	Grants Pass clay loam	0.0189	0.73	0.0183	0.71	
		0.0194	0.75	0.0194	0.75	+0.010
11674	Kirby gravelly loam	0.0197	0.76	0.0195	0.75	
		0.0202	0.78	0.0195	0.75	+0.020
11678	Esterly gravelly loam	0.0278	1.09	0.0292	1.13	
		0.0280	1.10	0.0292	1.13	−0.035
11679	Kirby loam	0.0211	0.82	0.0230	0.89	
		0.0210	0.82	0.0223	0.87	−0.060
11679*	Kirby loam	0.0220	0.86			
		0.0212	0.83			−0.035
11684	Tokay clay loam	0.0097	0.37	0.0092	0.36	
		0.0097	0.37	0.0088	0.34	+0.020
11682	Holland coarse sandy loam	0.0556	2.16	0.0555	2.15	
		0.0544	2.12	0.0551	2.13	0.000
11693	Colman loam	0.0485	1.88	0.0482	1.87	
		0.0472	1.84	0.0471	1.84	+0.005

* Rerun at a lower temperature. See text.

subsurface soils is characteristic because of differences between surface and subsurface soils in their content of organic matter. Fusions in the silica crucibles did not check with each other as closely as fusions in the J. Lawrence Smith platinum crucibles. But, inasmuch as duplicate fusions in the silica crucibles check as closely as fusions in the J. Lawrence Smith nickel with fusions in J. Lawrence Smith platinum crucibles, we believe we are warranted in claiming for the silica crucibles, used as we have used them with the muffle furnace, at least equal dependability. Again, inasmuch as nothing is to be gained by carrying out the analytical work on the soils with greater accuracy than obtains in sampling soil types in the course of soil survey work, the substitution of the silica crucibles for both nickel and platinum is not, we believe, open to serious adverse criticism. The combined use of these crucibles and the electric muffle furnace, carefully regulated as to temperature, makes our potash determinations much less costly; the sacrifice in accuracy, if any, can be but trifling in comparison.

As many as 24 fusions have been made in one crucible, during which time it has lost in weight a total of 232 mgm. It is, of course more fragile than at the beginning of its use, but it is still good for many more determinations. We have not gotten longer service from our nickel crucibles of the J. Lawrence Smith type. Indeed so well have these crucibles stood up under the treatment given them that we thought it worth while to experiment still further with the idea of securing eventually a combination of electric heat and silica crucibles of the J. Lawrence Smith type fully as dependable as are the time-honored J. Lawrence Smith crucibles of platinum. The success attained is outlined in the concluding paragraphs of this paper.

For routine work in the analyses incident to the soil survey we are now using the electric muffle furnace and vitreosil crucibles, no. C6, with thoroughly satisfactory results. Briefly stated our procedure is as follows:

One-half gram of soil, ground to pass a 100-mesh sieve, is thoroughly mixed with 4½ gm. of a fusion mixture consisting of ammonium chloride and calcium carbonate in the proportion 1 to 8. When the charge has been transferred to the crucible, a hole is made in the mixture from top to bottom with a glass stirring rod close to the crucible wall. With the glass stirring rod, the fusion mixture is then tamped into this hole, causing it to spread out in a thin layer on the crucible bottom under the charge and finally to fill almost completely the hole itself. The crucible is then tapped lightly on the desk to distribute evenly the top of the charge. Thus prepared it is ready for the muffle. From 4 to 8 crucibles in duplicate make up a single run. They go into the muffle at room temperature and are removed promptly when reading 32 on the millivoltmeter (855°C.) is reached. The time required for fusion is approximately 1 hour and 20 minutes. When the crucibles are sufficiently cool, enough water is added to cover the fused mass in each and they are set aside for a couple of hours for slaking and digestion. Very frequently the fusions are timed so that slaking and digestion take place over night. Filtration and thorough washing of the digested material usually results in potash solutions of approximately 600 cc. From here on the procedure is practically that of the Association of Official Agricultural Chemists, the potassium being weighed in porcelain gooch crucibles as K_2PtCl_6.

USE OF J. LAWRENCE SMITH SILICA CRUCIBLES

The crucibles thus far tried out were made for us according to specifications. They are of transparent vitreosil, 10 cm. in length, 2 cm. in diameter at the top

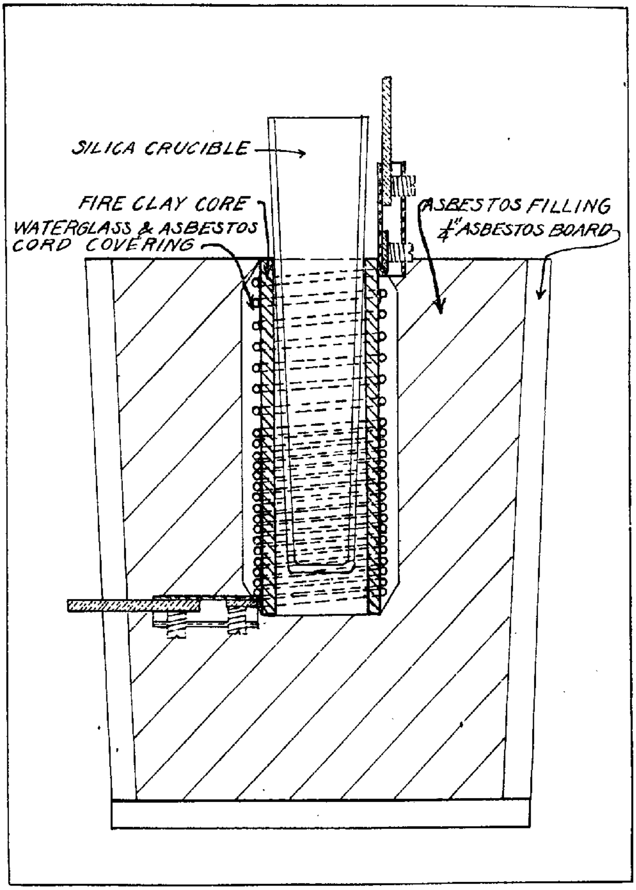

FIG. 3. CROSS-SECTION THROUGH AN ELECTRIC FURNACE ESPECIALLY DEVISED FOR USE WITH SILICA CRUCIBLES OF THE J. LAWRENCE SMITH TYPE

and taper to a diameter of 1.8 cm. at the bottom. Attempts to make fusions in them with the gas flame were not successful. The potash determinations in all cases were erratic and not dependable because of imperfect fusions. Obviously their use with the muffle furnace, if that were otherwise practicable, would

defeat the very purpose for which this type of crucible was designed. A simple electric furnace was finally devised that solved for us very satisfactorily the problem of heating these crucibles. In it the crucible stands erect. The lower end containing the charge may be heated to bright redness with no danger whatever of overheating the upper portion. In its crude state the furnace consists of a box of ¼-inch asbestos board 4 inches by 4 inches by 5 inches. The heating element is a discarded coil from an electric heater rewound to meet our purpose and protected from the air by several coats of a mixture of waterglass and finely divided asbestos fiber. This coil stands in the center of the box. The remaining space is tightly packed with asbestos fiber. From figure 3 it will be noticed that the crucible protrudes a full 2½ cm. from the clay cylinder

TABLE 6

Potash determinations from fusions made in a silica crucible of the J. Lawrence Smith type with electric furnace heat and in a platinum crucible of the J. Lawrence Smith type, gas heated

LABORATORY NO.	SOIL TYPE	FUSION IN THE SILICA CRUCIBLE		FUSION IN THE PLATINUM CRUCIBLE		DIFFERENCE
		K_2PtCl_6	K_2O	K_2PtCl_6	K_2O	
		mgm.	*per cent*	*mgm.*	*per cent*	*per cent*
11738	Peat (surface)	14.0	0.543	14.0	0.543	0.000
11753	Whiteson silt loam (subsurface)	11.4	0.442	11.4	0.442	0.000
11757	Aiken silt loam (subsurface)	19.5	0.754	19.4	0.749	+0.005
11764	Aiken clay loam (subsurface)	14.8	0.574	15.2	0.589	−0.015
11771	Yamhill loam (subsurface)	29.8	1.155	29.9	1.160	−0.005
11773	Gale loam (subsurface)	31.9	1.235	31.0	1.207	+0.028

of the coil. The diameter of the cylinder should be such as to permit the crucible to rest with its top protruding not over 1 cm. It would be better still if the clay cylinder were replaced with a tapering tube with top and bottom diameters just great enought to allow the silica crucible to slide easily into it. The heating element is connected in series with a rheostat to insure for it a safe working temperature. A box of this size could easily be made to accommodate two heating coils, and a battery of, say, four or five similarly contrived furnaces would greatly facilitate potash determinations when fusions in crucibles of the J. Lawrence Smith type are thought to be necessary. Fusions in nickel or platinum crucibles, of course can be made in the same type of furnace, thus in any event freeing the analyst from troubles incident to an unsatisfactory gas supply.

The cost of the silica crucibles of the J. Lawrence Smith type is approximately the same as that of nickel crucibles of the same size and type. Our conviction is that they will last much longer. Future orders, however, will be for the glazed vitreosil. At present prices a battery of 12 silica crucibles can be secured for the price of one small platinum crucible. In the silica crucibles 1-gm. charges can be fused; a 0.5-gm. charge is the limit for the platinum. The silica crucibles, however, will give way much sooner than will the platinum.

In table 6 are shown analytical data for potash secured by the combined use of a transparent silica crucible of the dimensions previously given and the crude electric furnace illustrated in figure 3, and by fusion in a J. Lawrence Smith crucible of platinum. All fusions were of 0.5-gm. charges.

SUMMARY AND CONCLUSIONS

Nickel crucibles of the J. Lawrence Smith type are much cheaper than platinum and have been used very satisfactorily to a certain extent in soil analysis for the fusions incidental to potash determinations. They are, however, comparatively short-lived, and, if heated with gas in the manner customary with crucibles of this type, potash determinations are relatively slow and costly. Our desire to reduce to the minimum the use of an unsatisfatory gas supply, to lessen the cost and to speed up generally the analytical work involved each year in the soil survey of this state, led to a try-out of electric furnace heat and crucibles of silica for the fusions necessary in potash work.

We find that an electric muffle furnace and silica crucibles of the ordinary shapes used for ignitions can be used for the fusions with highly satisfactory results, provided certain limits of temperature are observed. To insure perfect fusions, the muffle must reach a temperature of 812°C., and, to avoid loss of potash by volatilization, its temperature must not exceed 855°C. Fusion at the lower temperature for approximately 90 minutes, and prompt removal of the crucibles from the muffle when its temperature reaches 855°C., give practically the same results. The first is probably the safer procedure; the second is quicker. A thermo-couple connected to a millivoltmeter, makes a very satisfactory temperature indicator. The number of fusions one can make at the same time is limited only by the size of the muffle. We find it practicable to make as many as eight in duplicate. Lessened expense follows from the substitution of electricity for gasoline gas, replacement of expensive platinum by relatively cheap crucibles and in the larger number of samples one analyst can handle in a unit of time. The sacrifice in accuracy is inappreciable for practical purposes.

A number of fusions of soil for potash have been made in silica crucibles of the J. Lawrence Smith type. When heated with gas the fusions were never perfect and the resulting figures for potash not at all dependable. When heated in an electric furnace of special design, fusions in these crucibles were perfect and resulting potash determinations were equally satisfactory with those made from fusions in platinum crucibles of the same type. For this combination of crucible and furnace careful regulation of temperature is not essential. The simple construction of the furnace described makes possible and practicable its

THE EFFECT OF GYPSUM ON SOIL REACTION

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INTRODUCTION

Gypsum has been used as a fertilizer for many years, and has often proven to be valuable especially when applied to small grains and clovers. The early investigators claimed that gypsum increased crop yields for a time, but its continued use failed to maintain those increases, and as it contained no nitrogen, phosphorus, or potassium, it was regarded as a soil stimulant. Several more recent investigations have indicated that the value of gypsum is probably due to its sulfur content, and to the indirect action it may have on other soil constituents. Recently many soils have been found to be deficient in sulfur, one of the essential plant-food elements, and sulfur requirements of certain crops are apparently much greater than formerly supposed. Since gypsum supplies this necessary element in an available form it may play an important rôle in soil fertility in those regions where sulfur is needed.

It is generally conceded that gypsum, $CaSO_4 . 2H_2O$, cannot replace lime as a means of correcting soil acidity, but there seems to be some difference of opinion as to whether or not when applied to soils it tends to made them acid. Gypsum is a neutral salt and its dissociation in pure water produces hydrogen and hydroxyl ions of equal concentration. It is quite natural to expect it to act in a similar way when dissolved in the soil solution. However, through crop production or bacterial activities there may be a utilization of either the sulfate or the calcium ion, leaving one or the other free to react with the soil constituents in such a way as either to increase or decrease the acidity of the soil. There is also the possibility of a chemical reaction, the calcium combining with the insoluble soil acids, leaving the readily soluble sulfate ion which theoretically may cause a certain amount of acidity. The object of this work was to determine as far as possible by laboratory experiments the effect of gypsum on soil reaction.

HISTORICAL

In reviewing the literature on the effect of gypsum on soil reaction, the investigations reported may well be considered from two viewpoints: first,

[1] The writer wishes to make acknowledgments to Dr. P. E. Brown and Prof. H. W. Johnson for helpful suggestions and criticisms offered during the progress of this work.

experiments dealing with gypsum itself; and, second, experiments showing the effect of acid phosphate on acidity. Acid phosphate contains about 60 per cent gypsum and consequently any effect of this fertilizer on acidity may indirectly be interpreted as due at least partly to the presence of gypsum.

In 1910 Gardner and Brown (9), using the Veitch method, tested the lime requirement of the general fertilizer plots of the Pennsylvania station. Plots 13 and 33 received gypsum at the rate of 320 pounds per acre every two years. After 30 years the average of all the check plots had a lime requirement of 172 pounds of CaO per acre, as compared with 146 pounds for the gypsum plots Frear (8) concluded from these results that where land plaster was used the land was no more acid than where no fertilizer at all was applied.

Ames and Schollenberger (1) used the Hopkins potassium nitrate and the vacuum method and found that gypsum applied with manure decreased the acidity of the soil.

Lipman (19) stated that gypsum possesses no alkalinity and therefore will not be of any assistance nor act as a corrective to a sourness or acidity in soils.

Skinner and Beattie (26) used a number of square-rod plots to test the effects of fertilizers and soil amendments on soil acidity. $CaSO_4$ was used at the rate of 500 pounds per acre each year and after 5 years the plots were tested for lime requirement by the Veitch method. They concluded that the soil in each plot to which $CaSO_4$ had been added was more acid than the untreated plot growing the same crop. The average lime requirement for the $CaSO_4$ plots in excess of the check plots was 343 pounds of $CaCO_3$ per acre.

Singh (25), using pot cultures, tested the soil (after cropping) by the modified Tacke method and found that the acidity was increased by the application of gypsum, the larger amount giving the greatest increase.

Connor (7) tested gypsum as a means of correcting soil acidity on two types of very acid soils. The rate of application was 2 tons of gypsum per million pounds of soil. The gypsum treatment showed an actual decrease in acidity when compared with the check pots, the average decrease for the two soils being 580 pounds of $CaCO_3$ per million pounds of soil by the Hopkins potassium nitrate method, and 937 pounds by the Jones calcium acetate method.

Veitch (29) tested the lime requirement of the soil from a number of the plots in the 5-year rotation experiments at Wooster, Ohio, and found that acid phosphate had slightly reduced the acidity of the soil.

Brooks (4) concluded from his work that the use of an acid phosphate (dissolved bone black), at least had not increased the necessity for lime and, on the contrary, it seemed that the bone black had reduced the acidity.

Conner (6), testing the experimental plots at Purdue, observed that soils treated with acid phosphate for 20 years showed less acidity than soils that had received no treatment.

Ames and Schollenberger (1) claim that acid phosphate did not have any important influence on the depletion of the soil's supply of bases.

Bear and Salter (2) tested the residual effects of fertilizers and concluded that the use of manure or fertilizers (with the exceptionof K_2SO_4) had a tendency to decrease the acidity of the soil.

More recently Plummer (22) studied soil reaction by means of the hydrogen electrode. The plots to which acid phosphate had been added did not show any greater hydrogen ion concentration than the ones used as controls. These plots had received rather heavy annual applications of this fertilizer for the past 15 years, the total amount being over 3,000 pounds per acre.

Some of the above-mentioned investigations show a difference of opinion regarding the effects of gypsum on soil acidity. However, in every case where it is claimed that gypsum produced acidity the results obtained by the particular lime-requirement method employed are so small as to be well within the limit of experimental error, since it is doubtful whether any lime-requirement method in use at the present time is accurate to 400 pounds of calcium carbonate per acre. All the work reported relating to the effect of acid phosphate on acidity shows conclusively that this fertilizer tends to decrease rather than increase soil acidity.

EXPERIMENTAL

Two laboratory experiments were planned to determine:

(a) The effect of gypsum on an acid soil, a neutral soil, and a basic soil;

(b) The effect of gypsum on a soil with different degrees of acidity.

Experiment I

The three soils used in the first experiment were obtained from different locations in the Wisconsin drift soil area and all would be classed as Carrington loam.

The acid soil had a lime requirement of 2260 pounds of $CaCO_3$ per acre as determined by the modified Tacke method (27).

The neutral soil gave a negative test for acidity by the modified Tacke method and the Truog qualitative test.

The basic soil was obtained from a typical Iowa alkali spot and was very high in calcium bicarbonate.

The soils were air-dried and 2500 gm. of each soil was placed in 6 1-gallon pots. Treatments were made as follows:

Pot 1. Check
Pot 2. 100 pounds gypsum per acre
Pot 3. 200 pounds gypsum per acre
Pot 4. 500 pounds gypsum per acre
Pot 5. 1000 pounds gypsum per acre
Pot 6. 2000 pounds gypsum per acre

The gypsum was mixed with the entire contents of the pots and distilled water added to bring all the soils to their optimum moisture content. The pots were then weighed and covered loosely with a wooden block to prevent

rapid evaporation. The pots were kept in the laboratory at ordinary room temperature. A record was kept of the amounts of soil removed by sampling and every 2 weeks during the experiment the pots were reweighed and the moisture content maintained by additions of distilled water to weight. This experiment was begun March 3, 1920, and discontinued January 10, 1921.

The soil used in the second experiment was the same neutral soil as used in experiment I.

A preliminary test was made to compare the effect of gypsum on the hydrogen-ion concentration of a soil, made acid by treatment with H_2SO_4 and HCl. For this test 1000 gm. of neutral soil was used with each treatment and the hydrogen-ion concentration of a suspension from each treated soil determined. Another test was made to compare the effect of gypsum on the hydrogen-ion concentration of pure water made acid by treatments with H_2SO_4 and HCl, and for this test 200 cc. of double distilled water was used with each treatment. The acid treatments were calculated to give the same hydrogen-ion concentration. The tests may be best understood by the treatments as shown in table 1 which also gives the results.

TABLE 1

Hydrogen-ion concentration of a soil suspension and of pure water made acid by treatments with sulfuric and hydrochloric acids

NUMBER	TREATMENT	SOIL SUSPENSION	WATER
		pH	*pH*
1	Check	7.13	7.60
2	H_2SO_4, 1 gm.	6.39	1.28
3	HCl, 1.93 gm.	6.39	1.18
4	H_2SO_4, 1 gm.; 2 gm. gypsum	6.28	1.21
5	HCl, 1.93 gm.; 2 gm. gypsum	6.25	1.15
6	H_2SO_4, 1 gm.; 4 gm. gypsum	6.23	1.18
7	HCl, 1.93 gm.; 4 gm. gypsum	6.35	1.15

It will be noticed from table 1 that upon the addition of 2 gm. of gypsum to the soil treated with H_2SO_4 and HCl, the hydrogen-ion concentration remained the same for each acid. With the 4-gm. gypsum treatment there was an increase in acidity for the H_2SO_4 amounting to 0.12 pH over the HCl treatment. In the case of the water solution the pH values were practically the same for 2-gm. and 4-gm. gypsum treatments, and also each acid showed the same pH value. The results of these tests clearly showed that sulfuric and hydorchloric acid acted in the same manner as far as the reaction in the soil and water was determined by the hydrogen electrode.

Accordingly, for the second experiment hydrochloric acid was used to make the soil of varying degrees of acidity, in order to eliminate the sulfate ion which would be produced from the sulfuric acid.

For this experiment 16 1-gallon glazed pots were filled with 4200 gm. of the air-dried neutral soil and treatments made as follows:

Pots 1 and 2. Check
Pots 3 and 4. 500 pounds gypsum per acre
Pots 5 and 6. 4000 pounds $CaCO_3$ per acre
Pots 7 and 8. 4000 pounds $CaCO_3$ per acre plus 500 pounds gypsum
Pots 9 and 10. HCl equivalent to 2-ton lime requirement
Pots 11 and 12. HCl equivalent to 2-ton lime requirement plus 500 pounds gypsum
Pots 13 and 14. HCl equivalent to 4-ton lime requirement
Pots 15 and 16. HCl equivalent to 4-ton lime requirement plus 500 pounds gypsum

The gypsum and $CaCO_3$ were mixed with the 4200 gm. of soil, and the hydrochloric acid was added to the distilled water required to bring the soil to the optimum moisture content. The pots were weighed and covered in the same manner as those in experiment 1. This experiment was begun July 30, 1920, and discontinued December 29, 1920.

Methods

Lime requirement. The method used for determining the lime requirement in these experiments was a modification of the one designed by Tacke (28). The original method consisted of treating the soil with an excess of calcium carbonate and water, bubbling hydrogen through the mixture and collecting the carbon dioxide expelled with an alkaline absorbent contained in a Pettenkofer absorption tube. The lime requirement was calculated from the carbon dioxide thus obtained.

Several modifications of this method have been used by investigators in this country. Wheeler, Hartwell, and Sargent (30) boiled the mixed soil and $CaCO_3$ solution in water and determined the carbon dioxide evolved.

Ames and Schollenberger (1) used a method similar to the one just described, the essential difference being the temperature employed. The boiling was conducted *in vacuuo* and the temperature need not exceed 50° C. This step was taken to reduce the decomposition of the organic matter by heat to a minimum.

Knight (18) mixed a weighed quantity of soil with precipitated calcium carbonate, added about 25 cc. of a neutral salt solution, boiled for a definite period and determined the carbon dioxide evolved by the Parr method.

The modification used in this work was the one described and used by Stephenson (27). A machine was used which held 8 300-cc. Kjeldahl flasks and 8 sodium-hydroxide towers. Twenty grams of soil, 2 gm. of calcium carbonate and 50 cc. of CO_2-free water were placed in the flasks and the carbon dioxide evolved during the shaking was absorbed in a solution containing 50 cc. 0.2 N NaOH and 100 cc. of CO_2-free water. A stream of CO_2-free air was constantly drawn through the system and continuous shaking employed for 5 hours. Determinations were made in duplicate, and blanks, without the calcium carbonate solution, were run for each determination. Results were obtained by double titration with phenolphthalein and methyl orange. Thus using a 20 gm. sample of soil and titrating against 0.04N acid, each cubic centimeter of

the titration represents a lime requirement of 400 pounds of calcium carbonate per two million pounds of soil. In the results the blank is always subtracted.

Acidity. In these experiments the acidity, or hydrogen-ion concentration, of the soil was measured by the hydrogen electrode. The possibilities of this method of studying soil reactions have been realized only during comparatively recent years. Gillespie (10) used the electrometric and colorimetric methods and determined the hydrogen-ion concentrations of a number of soils of different types. Sharp and Hoagland (24) studied the hydrogen-ion concentration in various soil suspensions and in soil extracts, and noted the effect of certain factors on the hydrogen-ion concentration. Hoagland and Sharp (15) found that the hydrogen-ion concentration of soil suspensions of acid soils was not markedly affected by increasing the CO_2 content up to 10 per cent.

Gillespie and Hurst (12, 13) studied the hydrogen-ion concentration of two soil types, the Caribou loam and Washburn loam, and showed a correlation between hydrogen-ion concentration and the occurrence of the potato scab. Martin (21) studied the relation of the potato-scab organism to soil acidity as measured by the hydrogen-ion concentration, and also for determining the relation of hydrogen-ion concentration of extracts from soils to which varying amounts of sulfur had been added.

Plummer (22) used the hydrogen electrode method in comparing hydrogen-ion concentrations of a number of untreated soil samples and also of a few samples of soil treated with different fertilizers. Kappen and Zapfe (17) measured the hydrogen-ion concentration of peat soils and of peat and humus-forming plants.

Gillespie (11) proposed a colorimetric method for the determination of hydrogen-ion concentrations without buffer mixtures. His indicators were chosen from the series of Clark and Lubs (5). Wherry (31, 32) described a set of six indicators suitable for the study of soil reactions in the field, and gave detailed instructions for making the test.

Several investigators have compared the hydrogen-ion concentration of soil extracts with certain lime-requirement methods. Knight (18) found that the vacuum method approached nearer to the lime requirement as shown by the hydrogen electrode than any of the five methods studied. The Veitch method showed results considerable lower.

Blair and Prince (3) observed a close correlation between the hydrogen-ion concentration of the soil extract and the lime requirement by the Veitch method. Soils having a pH value of 6.7 or over were alkaline by the Veitch method. Joffe (16) found that soils having a pH value 6.6 to 6.8 before evaporation indicated that the end point of the lime requirement by the Veitch method had been reached.

The apparatus used in determining the hydrogen-ion concentration in this work was similar to that described by Sharp and Hoagland (24) which is a modification of Hildebrand's (14) apparatus. A normal calomel cell and an

accurate Weston voltmeter were used in the present work. Results could be obtained with this apparatus accurate to within 0.05 pH. Fifteen grams of soil and 30 cc. of double distilled water were used in the manipulation of the test, duplicate determinations being made for each sample. The voltmeter readings were transformed into pH values from the tables prepared for this purpose by Schmidt and Hoagland (23).

Residual Carbonates. Residual carbonates were determined by the method of MacIntire and Willis (20). The same apparatus was used as that described for determining the lime requirement. Twenty grams of air-dry soil, and 50 cc. of 1 to 15 phosphoric acid solution, prepared by diluting 85 per cent H_3PO_4 with CO_2-free water, were placed in the flasks. The flasks were constantly shaken for 30 minutes. A current of CO_2-free air was drawn through the system at a very slow rate for the first 10 minutes. The second 10 minutes the rate was slightly increased and during the last 10 minutes the air was drawn through fairly rapidly. The carbon dioxide evolved was absorbed in a sodium hydroxide solution, and results were obtained by double titration with phenolphthalein and methyl orange.

Discussion of results

Twenty-gram samples of the wet soils were taken from the pots of the first experiment at different times and tested for their lime requirement by the Tacke method as described. Correction was made for moisture in the samples and the results were figured in pounds of $CaCO_3$ per 2,000,000 pounds of soil. It may be well to call attention at this point to the accuracy of this method as it was used in this work. Duplicate determinations usually checked to within 200 pounds of $CaCO_3$ per acre, but occasionally differences were obtained as much as 400 pounds of $CaCO_3$. Consequently it would be safe to say the method is accurate only to 400 pounds of $CaCO_3$ per acre.

The results for the three soils, acid, neutral, and basic, are grouped under table 2. It will be seen from the data presented for the acid soil, that the first determinations made on March 18, showed wider variations between the check pot and the gypsum treatments than at any other date. The 200-pound gypsum treatment showed a decrease of 321 pounds of $CaCO_3$ in lime requirement when compared with the check. The 2000-pound gypsum treatment showed an increased lime requirement over the check amounting to 554 pounds of $CaCO_3$. This amount would indicate a slight acidity caused by the gypsum, but the average of the four determinations for the 2000-pound treatment is only a little over half this amount. While the lime requirements of the variously treated pots do not agree very closely at each sampling, there is a rather close agreement for the average. The gypsum treatments up to and including 1000 pounds per acre show practically the same lime requirement as the check pot. There is an increase of 243 pounds of $CaCO_3$ in lime requirement for the 2000-pound gypsum treatment over the check pot, which would indicate a slight increase in acidity if the method was accurate to 100

pounds of $CaCO_3$ per acre, but when the accuracy is really about 400 pounds no increase in acidity can be concluded.

Before treatment the neutral soil showed no lime requirement by the Tacke test. After treatment the check soil showed a lime requirement of 250 pounds. The pots receiving 100 and 200 pounds of gypsum showed a smaller lime

TABLE 2

Lime requirement by the Tacke method of three soils treated with different amounts of gypsum

Acid soil

POT NUMBER	TREATMENT PER ACRE	DATE SAMPLED				AVERAGE
		March 18	June 18	November 17	January 10	
		lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*
1	Check	2278	3183	3271	3270	3000
2	Gypsum 100 lbs.	2570	3271	3037	3066	2986
3	Gypsum 200 lbs.	1957	3154	3592	3416	3029
4	Gypsum 500 lbs.	2480	3271	3592	3153	3124
5	Gypsum 1000 lbs.	2366	3329	3388	3550	3158
6	Gypsum 2000 lbs.	2832	3329	3329	3601	3243

Neutral soil

POT NUMBER	TREATMENT PER ACRE	DATE SAMPLED			AVERAGE
		March 17	June 16	November 15	
		lbs.	*lbs.*	*lbs.*	*lbs.*
1	Check	390	90	270	250
2	Gypsum 100 lbs.	330	150	60	180
3	Gypsum 200 lbs.	Alkali	60	Alkali	20
4	Gypsum 500 lbs.	120	420	600	380
5	Gypsum 1000 lbs.	540	180	330	350
6	Gypsum 2000 lbs.	330	390	480	400

Basic soil

POT NUMBER	TREATMENT PER ACRE	DATE SAMPLED		
		March 19	June 24	November 24
1	Check	Trace	Alkali	Alkali
2	Gypsum 100 lbs.	Trace	Trace	Alkali
3	Gypsum 200 lbs.	Alkali	Alkali	Alkali
4	Gypsum 500 lbs.	Alkali	Alkali	Trace
5	Gypsum 1000 lbs.	Alkali	Alkali	Trace
6	Gypsum 2000 lbs.	Alkali	Alkali	Alkali

requirement than the check pot, but the pots receiving 500, 1000, and 2000 pounds of gypsum showed increases in lime requirement over the check, amounting to 130, 100, and 150 pounds of calcium carbonate, respectively. These amounts, however, are too small to permit the conclusion that gypsum has any effect on acidity, since differences as much as 400 pounds of $CaCO_3$ could be within the limit of experimental error.

It would not be expected to get a lime requirement by the Tacke method on the untreated basic soil, but determinations were made along with the acid and neutral soil to see if the gypsum would cause sufficient acidity to show a lime requirement on this soil. In nearly every case an alkaline reaction was obtained but several tests show a trace of a lime requirement. It would appear from the results by the Tacke method shown in table 1 that gypsum had no effect on soil reaction, regardless of whether the soil was acid, neutral, or basic.

To determine the effect of gypsum on the soil reaction, samples were taken from the pots in experiment 1 in 3 days, 10 days, 30 days, 5 months and 10 months after treatment for the determination of the hydrogen-ion concentration by the hydrogen electrode. The results for the three soils are summarized in table 3. The results are expressed in terms of pH values to simplify comparison. Each figure in the table represents the average of two determinations checking within 8 millivolts.

From a study of table 3 it will be noted that there is a striking uniformity in the results reported for the different samplings, and therefore in discussing these data only the average of the five determinations will be considered.

The first four treatments of the acid soil, including the check, showed an average pH value of 5.5 indicating no production of acidity due to applications of gypsum to 500 pounds per acre. The 1000-pound gypsum treatments showed an increase in acidity of 0.21 pH over the check, and the 2000-pound treatment showed an increase of 0.28 pH. Since the apparatus used for these determinations was accurate to 0.05 pH, these differences noted are large enough to conclude that gypsum does produce very slight acidity in an acid soil when applied at the rate of 1000 or 2000 pounds per acre. Comparing the 1000-pound gypsum treatment with the 2000 pounds we note the amount of acidity produced was not in proportion to the amount of gypsum applied.

The average of the first four treatments of the neutral soil showed almost identical pH values. The last two treatments, 1000 and 2000 pounds of gypsum, showed slight increases in acidity over the check of 0.14 pH and 0.27 pH, respectively. In the case of the neutral soil the amount of acidity caused by the 1000-pound treatment with gypsum was smaller by 0.07 pH than the same treatment of the acid soil. The same amount of acidity was produced by the 2000-pound gypsum treatment in both the acid and neutral soils. The acidity of the 2000-pound treatment of the neutral soil is just twice that of the 1000-pound treatment, showing that the increase in acidity was directly proportional to the amount of gypsum added. This was not true with the acid soil.

The average pH values were the same for the first four treatments for the basis soil. The 1000-pound gypsum treatment showed an increase in acidity of only 0.09 pH, and the 2000-pound treatment showed an increase of only 0.12 pH as compared with the check. Thus the pH values for the 1000 and the 2000-pound treatments were approximately the same, since the 0.03

pH difference is within the limit of experimental error. There being a difference of only 0.09 pH between the check and the 1000-pound treatment, it is safe to conclude that the heavier applications of 1000 and 2000 pounds of gypsum are less apt to cause acidity in a basic soil than they are in an acid soil or a neutral soil.

TABLE 3

Hydrogen-ion concentration of three soils treated with different amounts of gypsum

Acid soil

POT NUMBER	TREATMENT PER ACRE	DATE SAMPLED					AVERAGE
		March 6	March 13	March 26	August 6	January 5	
		pH	*pH*	*pH*	*pH*	*pH*	*pH*
1	Check................	5.69	5.68	5.57	5.35	5.47	5.55
2	Gypsum 100 lbs........	5.78	5.64	5.52	5.29	5.30	5.50
3	Gypsum 200 lbs........	5.56	5.66	5.51	5.35	5.30	5.47
4	Gypsum 500 lbs........	5.52	5.63	5.52	5.35	5.44	5.49
5	Gypsum 1000 lbs......	5.35	5.52	5.46	5.20	5.19	5.34
6	Gypsum 2000 lbs......	5.29	5.35	5.32	5.19	5.19	5.27

Neutral soil

POT NUMBER	TREATMENT PER ACRE	DATE SAMPLED					AVERAGE
		March 6	March 12	March 25	August 5	January 6	
		pH	*pH*	*pH*	*pH*	*pH*	*pH*
1	Check................	7.06	7.05	6.96	7.05	7.15	7.05
2	Gypsum 100 lbs.......	7.03	7.03	6.98	7.05	7.06	7.03
3	Gypsum 200 lbs.......	7.03	6.99	6.91	7.03	6.98	6.99
4	Gypsum 500 lbs.......	6.96	7.01	6.91	6.99	7.05	6.98
5	Gypsum 1000 lbs......	6.88	6.88	6.89	6.94	6.99	6.91
6	Gypsum 2000 lbs......	6.71	6.72	6.71	6.88	6.88	6.78

Basic soil

POT NUMBER	TREATMENT PER ACRE	DATE SAMPLED					AVERAGE
		March 7	March 15	March 27	August 7	January 7	
		pH	*pH*	*pH*	*pH*	*pH*	*pH*
1	Check................	7.89	8.03	8.01	8.04	8.04	8.00
2	Gypsum 100 lbs.......	7.87	7.92	8.01	8.04	8.18	8.00
3	Gypsum 200 lbs.......	7.89	7.92	8.01	8.03	8.04	7.98
4	Gypsum 500 lbs.......	7.89	7.98	7.89	7.99	8.06	7.96
5	Gypsum 1000 lbs......	7.76	7.82	7.89	8.01	8.06	7.91
6	Gypsum 2000 lbs......	7.76	7.86	7.76	7.89	8.04	7.88

It is interesting to note in the case of the acid soil that the hydrogen-ion concentration for the check soil remained almost constant for the first month after the experiment was started. Five months later there was a slight increase in acidity amounting to a little over 0.2 pH. Also, with the pots treated with gypsum there was the same tendency for a constant hydrogen-ion con-

centration for the first month after treatment, and 5 months later a development of acidity. These data would indicate that the soil organisms were active during the continuance of the experiment and were able to aid in any possible chemical reaction with the gypsum and other soil constituents.

To summarize the results of table 3, it may be concluded that gypsum applied in amounts up to and including 500 pounds per acre does not increase or decrease the hydrogen-ion concentration of an acid soil, a neutral soil, or a basic soil high in calcium bicarbonate. When gypsum is applied in amounts as high as 1000 and 2000 pounds per acre there is a slight increase in hydrogen-ion concentration of an acid soil and a neutral soil, but the increase is very slight in the basic soil. These treatments, however, are much larger applications than are used under actual field conditions.

Experiment II

Experiment II was planned to determine the effect of gypsum on the reaction of a neutral soil treated with calicium carbonate and hydrochloric acid to give different degrees of acidity.

The soils in this experiment were sampled at three different times and tested for their lime requirement. The results of these tests, expressed in pounds of $CaCO_3$ per acre, appear in table 4. Determinations were made in duplicate for each treatment and each figure in the table represents the average of four determinations.

Considering first the data presented in table 4 for the neutral soil with and without gypsum, it will be noted that each test showed an increased lime requirement for the gypsum treatment over the untreated soil. The average lime requirement for the gypsum treatment in excess of the untreated soil, however, was only 230 pounds of $CaCO_3$ per acre, which is too small an amount to consider important, because the range of the experimental error for the method was 400 pounds of $CaCO_3$ per acre.

The soil treated with calcium carbonate showed a trace of lime requirement with and without the gypsum. This statement is in accord with the conclusion drawn from the effect of gypsum on the basic soil used in experiment I.

The soil treated with hydrochloric acid to make a 2-ton lime requirement also showed an increased lime requirement for the gypsum treatment in excess of the untreated soil. In the case of the test made on August 14, the gypsum treatment showed a lime requirement of 765 pounds of $CaCO_3$ in excess of the untreated soil, but the average lime requirement for the three tests in excess of the untreated soil was 370 pounds of $CaCO_3$, which also is within the 400 pound limit.

The soil treated with hydrochloric acid to make a 4-ton lime requirement showed an average lime requirement of 280 pounds of $CaCO_3$ for the gypsum treatment in excess of the soil without gypsum. This amount likewise is not sufficient to justify the claim that any production of acidity was due to the presence of gypsum.

The residual limestone was determined in the soils of experiment II to note whether the production of acidity caused by the gypsum would decrease the amount of carbonates present in the soil. The average results of the three determinations are included in table 4. Comparing the results of the lime-requirement tests with those of the residual limestone, we see in each case, except one, that where there is an increase in lime-requirement, apparently due to the gypsum, there is a gain in residual limestone. But these differences, as in the case of the average lime-requirement tests, are very small and well within the limit of experimental error. They do show, however, that the apparent acidity caused by the gypsum does not decrease the supply of soil carbonates, and this fact would indicate that gypsum does not increase the acidity.

TABLE 4

Lime requirement by the Tacke method and residual limestone of a neutral soil treated to vary in different degrees of acidity

POT NUMBER	TREATMENT PER ACRE	LIME REQUIREMENTS			AVERAGE	
		August 4	August 14	December 1	Lime requirement	Residual carbonates
1–2	Check, (neutral soil)	375	315	15	235	1496
3–4	Gypsum, 500 lbs.	810	450	135	465	1633
5–6	$CaCO_3$, 4000 lbs.	Trace	Trace	Trace		4829
7–8	$CaCO_3$, 4000 lbs., plus gypsum, 500 lbs.	Trace	Trace	Trace		4886
9–10	HCl equivalent to 2 tons lime requirement	1965	1500	915	1460	1533
11–12	HCl equivalent to 2 tons lime requirement plus gypsum, 500 lbs.	2220	2265	1005	1830	1833
13–14	HCl equivalent to 4 tons lime requirement	3990	5085	2100	3725	1346
15–16	HCl equivalent to 4 tons lime requirement plus gypsum, 500 lbs.	4170	6120	1725	4005	1276

It seems strange to have a lime requirement of varying amounts up to 4000 pounds of $CaCO_3$ per acre and still have residual limestone present. This condition may possibly be explained by the nature of the methods involved and the particular soil which was used in this experiment. This neutral soil undoubtedly contained small particles of limestone, the outer surfaces of which were hard and weathered, which caused them to be more or less resistant to the reaction that takes place in the lime-requirement determination. On the other hand, the constant agitation of the soil with the phosphoric acid, as used in the determination of carbonates, would permit the complete decomposition of all limestone particles and hence show residual carbonates present.

It will be seen also from table 4, that residual carbonates were found even in the soils treated with hydrochloric acid to make a 2-ton and 4-ton lime requirement. The amount of carbonates present in the soil with the 2-ton lime requirement was practically the same as reported for the untreated soil. But

the amount of carbonates present in the soil with the 4-ton lime requirement is smaller by 200 pounds of $CaCO_3$ per acre than the soil with the 2-ton lime requirement. This would indicate that the weak dilution of acid used for the 2-ton lime requirement treatment was not strong enough to decompose the limestone particles of the soil, and that the 4-ton requirement treatment was sufficient to decompose them only slightly.

Samples were drawn at three different times from the soils in experiment II and determinations made for hydrogen-ion concentrations. The results, expressed in terms of pH values, are given in Table V. Duplicate determinations were made of the soil in each pot, thus each figure in the table represents the average of four determinations.

A study of table 5 shows that the 500-pound treatment with gypsum for the neutral soil had the same average hydrogen-ion concentration as the

TABLE 5

Hydrogen-ion concentration of a neutral soil treated to varying degrees of acidity

POT NUMBER	TREATMENT PER ACRE	DATE SAMPLED			AVERAGE
		August 2	August 16	December 13	
		pH	*pH*	*pH*	*pH*
1– 2	Check (neutral soil)	7.05	7.54	7.02	7.20
3– 4	Gypsum, 500 lbs.	7.05	7.45	7.05	7.18
5– 6	$CaCO_3$, 4000 lbs.	7.38	7.89	7.54	7.60
7– 8	$CaCO_3$, 4000 lbs.; plus gypsum, 500 lbs.	7.39	7.81	7.47	7.58
9–10	HCl equivalent to 2 tons lime requirement	5.50	4.47	6.27	5.41
11–12	HCl equivalent to 2 tons lime requirement plus gypsum, 500 lbs.	5.63	4.85	6.22	5.56
13–14	HCl equivalent to 4 tons lime requirement	4.87	3.85	5.70	4.80
15–16	HCl equivalent to 4 tons lime requirement plus gypsum, 500 lbs.	4.67	4.13	5.80	4.86

same soil without gypsum. This condition was also true with the soil treated with 4000 pounds of $CaCO_3$ per acre.

Considering next the soil treated with hydrochloric acid to make a 2-ton lime requirement, we see that the determinations at each sampling for the soil with and without gypsum do not agree very closely. The average pH value of the three determinations for the soil without gypsum was 5.41, while the soil with gypsum showed an average pH value of 5.56. This showed a decrease in acidity amounting to 0.15 pH value for the gypsum treatment as compared with the soil without gypsum. However, this variation is not in agreement with the other soils under consideration, and because of the large differences obtained with different samplings, it cannot be concluded that gypsum decreased the acidity in this soil.

The soil treated with hydrochloric acid to make a 4-ton lime requirement also showed wide variations in pH values at different samplings. The first determination showed that the gypsum treatment increased the acidity

amounting to 0.2 pH as compared with the soil without gypsum. The next determination showed the reverse to be true. In this case gypsum decreased the acidity amounting to 0.28 pH. In the third determination the gypsum again showed a small decrease in acidity compared with the soil without gypsum. The average pH values of the three determinations were practically the same for the soil with and without gypsum.

It may be concluded from the results shown in table 5 that gypsum applied at the rate of 500 pounds per acre does not have any effect on the hydrogen-ion concentration of a neutral soil treated so as to vary in degrees of acidity.

While the results here reported are from laboratory experiments it is probable that they would apply in their essential points to field conditions. The writer has under way actual field experiments on several soil types in Iowa, and a study is being made to determine the effect of gypsum on soil reaction in the field. These results will be reported later.

COMPARISON OF RESULTS BY THE TACKE LIME REQUIREMENT METHOD WITH HYDROGEN-ION DETERMINATIONS

From the data presented in these experiments an opportunity is taken at this point to make a comparison of the results by the Tacke method with the hydrogen-ion determinations. By referring to tables 2 and 3 it will be noted in the case of the neutral soil that the average lime requirement for the first four treatments, including the check, was 207 pounds of $CaCO_3$ per acre, and the average hydrogen-ion concentrations of the same treatments with the same soil was 7.0 pH. The neutral point for the hydrogen-ion determinations in this work was 7.0 pH, while the neutral point for the Tacke method was from 0 to 400 pounds of $CaCO_3$ per acre. Thus a close correlation exists between the neutral points of the respective methods. No attempt was made to determine the lime requirement in this work by the hydrogen electrode, and consequently any further comparisons of the methods would be inconclusive and misleading because of different soil types and soil conditions.

CONCLUSIONS

1. Gypsum added in amounts from 100 to 2000 pounds per acre to an acid soil, a neutral soil, and a basic soil, did not increase or correct the acidity of the soil as shown by the Tacke lime-requirement method.

2. Gypsum applied at the rate of 100, 200, and 500 pounds per acre did not raise or lower the hydrogen-ion concentration of the soil as measured by the hydrogen electrode.

3. Excessive amounts of 1000 and 2000 pounds of gypsum per acre showed increases in acidity by the hydrogen electrode amounting to 0.21 pH and 0.28 pH, respectively, for the acid soil studied; 0.14 pH and 0.27 pH, respectively, or the neutral soil studied; and 0.09 pH and 0.12 pH, respectively, for the basic soil.

4. Gypsum applied at the rate of 500 pounds per acre to a neutral soil made to vary in degrees of acidity by additions of HCl and $CaCO_3$ had no effect on the hydrogen-ion concentration of the soil, and did not show sufficient lime requirement by the Tacke method to permit of the conclusion that gypsum had any effect on acidity.

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EXPERIMENTS WITH COMMON ROCK SALT: I. EFFECT ON ASPARAGUS[1]

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Sodium chloride or its elements are present in almost all plants. It does not necessarily follow, however, that one of these elements is necessary, or even of value, to the plants. Investigators differ as to the value of common rock salt as a fertilizer for various agricultural crops. Salt in excess is injurious to plant growth and stops germination of the seed, but all plants and all seeds are not affected to the same extent by the action of salt. Some are easily injured by the application of salt, and when such a plant is noxious, salt may be made use of for its eradication.

EARLIER EXPERIMENTS WITH ASPARAGUS

Salt has long been considered one of the essential fertilizers in asparagus growing. With few exceptions the older books on practical gardening recommend a liberal use of salt. The greatest benefit was claimed to be on old plantings. This seems to correlate with the experiments recorded below. White (10) recommended a top-dressing of 2 pounds of salt to the square yard before growth had commenced, and Burr (2) advised the application of 2 quarts to the square yard. Brill (1) says, "Coarse salt may be used after the third year at the rate of 5 bushels to the acre along the rows, or three times this amount if sown broadcast." Oemler (6) says that ". . . . the natural habitat of asparagus being the seacoast, it is benefited by applications of common salt, which may be used in sufficient quantities to destroy weeds, particularly if the crop is some distance from the seashore." Rawson (7) recommends salt as "an excellent thing to apply for a dressing, for, although it does not act as a manure, it is a great help in keeping down weeds." Landreth (5) states that "20 bushels of salt to the acre may be used to advantage annually; 5 bushels to the acre stimulates the asparagus, which is a salt plant, and retards weeds." Hunn and Bailey (4) say that the practice of sowing salt on asparagus is almost universal. However, they found that beds which had never received a pound of salt were as productive as those which had received an annual dressing. Nevertheless, they recommend a salt dressing. Recent works on gardening either make no mention of salt

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in connection with asparagus growing, or express doubt about beneficial effects. Card (3), in reporting some experiments with asparagus and salt, says, "Salt has no beneficial effect and is injurious when used in considerable quantities." The results obtained by Walker (9) with five varieties of asparagus ". . . . showed a decided difference in favor of the salted area amounting to 13.5 per cent, or a difference of 103.32 pounds per acre for the planting in its third year. The difference was not confined to the spring growth. There was an increased vigor manifest throughout the summer, and furthermore a noticeably increased glaucous appearance in the salt plants." The application of salt, broadcasted at the rate of 1,000 pounds per acre, was given the previous summer.

EXPERIMENTAL WORK

The experiments reported herein were conducted on the horticultural farm at the New Jersey Agricultural Experiment Station. Plots were laid out in the middle of two fields on which asparagus had been growing for 2 years and 11 years, respectively. These fields, and subsequently the experimental plots, consisted of an equal number of rows running east and west. The younger plants stood 20 inches in the rows, with the rows 2 feet apart, while the older plants stood approximately 2 feet in the rows with the rows 3 feet apart. The area is level, and the soil is a Sassafras loam. The following diagram shows the experimental plots running in duplicate, and the applications of salt that were made.

PLANTS 2 YEARS OLD				PLANTS 11 YEARS OLD			
Check	150 lbs.	300 lbs.	500 lbs.	Check	150 lbs.	300 lbs.	500 lbs.
500 lbs.	300 lbs.	150 lbs.	Check	500 lbs.	300 lbs.	150 lbs.	Check

In addition to approximately 8 tons of cow manure and 4 tons of poultry manure the salt applications were made on the plots on the rows April 17, 1919, after a heavy rain. On April 13, 1920, salt was again applied, in addition to 8 tons of cow manure and 5 tons of poultry manure. The weather was rainy. Rainfall for the months April to September, 1919, at New Brunswick was 29.79 inches, and for the whole year was 52.41 inches, while the normal precipitation is about 45 inches.

From the younger beds but two cuttings were made, April 26 and May 2, 1919. From the older beds cuttings were made throughout the entire season from April 26 to June 18, 1919. The average weight per plant for the two plants is recorded in table 1.

As may be seen from these figures, the beds were quite uniform. The influence of the salt on the production of the stalks harvested can be considered as *nil*.

When the harvesting of the stalks ceases, a tall bushy growth develops which enables the plant to make greater use of the plant-food in the soil and store it up in the roots for stalk production the following season. The condition and vigor of the fall growth, therefore, has a direct bearing upon the yield and size of the stalks of the next season. In view of this growth as food storage, countings of plants and stems, and measurements of the latter,

TABLE 1

Average total weight of cuttings per plant of asparagus treated with salt

TREATMENT PER ACRE	YOUNG PLANTS	OLD PLANTS
lbs.	*gm.*	*gm.*
None	12.1	445
150	13.2	457
300	12.4	442
500	12.8	451

TABLE 2

Average number of stems, total length of stems, and average length of stems per plant, for two corresponding plots of 2-year-old asparagus plants

MEASUREMENTS		CHECKS			150 POUNDS OF SALT			300 POUNDS OF SALT			500 POUNDS OF SALT		
Number	Date	Number of stems	Total length of stems	Average length of stems	Number of stems	Total length of stems	Average length of stems	Number of stems	Total length of stems	Average length of stems	Number of stems	Total length of stems	Average length of stems
			cm.	*cm.*		*cm.*	*cm.*		*cm.*	*cm.*		*cm.*	*cm.*
1	May 9	1.30	48.5	36.0	1.33	47.0	37.0	1.45	52.0	35.0	1.30	42.0	33.0
2	May 16	1.89	90.0	42.5	1.87	75.0	38.5	2.47	103.0	40.5	2.13	82.0	40.5
3	May 23	2.07	152.0	68.5	2.40	159.0	60.5	2.52	152.0	65.0	2.66	153.0	61.0
4	May 29	2.12	194.0	91.5	2.79	212.0	84.5	2.98	258.0	84.5	3.08	215.0	83.5
5	June 7	2.27	220.0	97.0	2.51	238.0	96.0	3.20	292.0	94.0	3.16	306.0	100.5
6	June 14	2.29	231.0	99.0	2.61	248.0	96.0	3.33	300.0	99.0	3.44	313.0	95.5
7	June 21	2.46	236.0	93.5	2.64	251.0	93.0	3.42	312 0	90.0	3.58	321.0	90.0
8	June 29	2.90	248.0	87.7	2.90	256.0	84.9	3.79	338.0	87.6	3.89	339 0	86.3
9	July 5	3.21	275.0	86.0	3.35	284.0	84.0	4.00	345.0	79.0	4.36	350.0	82.5
10	July 12	4.04	307.0	75.8	4.04	373.0	91.2	4.79	395.0	79.6	5.0	426.0	85.1
11	August 2	4.87			5.28			5.97			6.31		
12	August 23	5.73			5.97			6.57			6.88		
13	September 6	5.88			6.05			6.83			7.46		
Per cent increase..............					2.9	18.2		16.2	28.6		26.8	38.7	

were taken throughout the entire season, at intervals of from 7 to 10 days. An attempt was made to measure the thickness of the stalks, but was discontinued after these measurements had been correlated with the height of the stems. The average number of stems per plant, the total height of plants, and length of stems for the two corresponding plots of young plants are given in table 2. Counting and measuring was begun 7 days after the last cutting.

During the second half of the growing season the asparagus beetle did some damage which resulted in dead tips of the stalks.

The figures in table 2 show clearly the regular increase in the number of stems per plant and the corresponding total length of stems per plant, while the average length of the stems per plant reached a maximum in the middle of the season. Sprouts which appeared later in the season could not develop such a vigor as to reach the maximum height of the first stalks. The number of stems and the total length of stems per plant increased regularly in all series; but where applications of 300 and 500 pounds of salt per acre were made, the number of stems, and consequently the total length, was considerably greater at the tenth measuring than that of the plants growing on the plots where 150 pounds of salt were applied. The average number of stems per plant on these plots were, respectively, 18.5 and 23.7 per cent greater than the average number of stems of the plants on the check plots. The total length per plant on these plots showed still greater differences, namely, 28.6 and 38.7 per cent more than the total length of the stems per plant on the check plots. Later in the season accurate measuring became extremely difficult on account of the entangling of the branches, but counting was continued though at greater intervals. At the end of the season, just before harrowing, the average number of stems of the plants on the plots which received respectively 150, 300 and 500 pounds of salt per acre in addition to the manure, as compared with the average number of stems on the check plots, showed an increase of 2.9, 16.2, and 26.8 per cent in the order named.

Countings and measurements also were made on the old plants but the measuring had to be discontinued later on. The entangling of the stem became so great that accurate measuring was impossible. Stems and plants were counted as usual. The average length of the stems in the earlier part of the growing season followed closely the number of stems, as was the case for the younger plants. According to observations made on July 25, August 10, and September 6, no difference in height could be seen though growth was probably more dense. The plants looked healthy on all plots until August 25 when the color of the plants began to change somewhat, on account of the advance of the season. On September 8 a number of plants showed yellowness and dried tips.

Three weeks after the last cutting was made the counting of the stems was begun, and was continued until September 6. The compiled data of the average number of stems per plant for the two corresponding plots are given in table 3.

The increase in top growth of the plants was regular on all plots. The number of stems at the beginning of the counting was the highest on the plots to which 500 pounds of salt was applied, and remained so until the end of the season. The number of stems at the end of the season was 2.1, 11.7, and 25.9 per cent over the check plots, for applications of 150, 300, and 500 pounds of salt per acre, respectively. These results were much more pro-

nounced than was expected. The average percentage of increase of the number of stems for the 2-year-old plants was very much the same as the average percentage of 11-year-old plants on all plots. They are given here with emphasis on the fact that they represent only a single season of countings.

The average weight per plant obtained by the cuttings of the young and of the old plants during the season of 1920 is given in table 4, together with the number of stalks graded according to the commercial way for marketing, which indicates the market value of the product as good, fair and poor stalks.

TABLE 3

Average number of stems per plant for two corresponding plots of 11-year-old asparagus plants in 1919

COUNTINGS MADE		NO TREATMENT	150 POUNDS SALT	300 POUNDS SALT	500 POUNDS SALT
Number	Date				
1	July 12	8.50	8.69	10.42	11.72
2	July 25	12.29	12.58	14.57	15.87
3	August 1	14.01	14.49	16.35	18.14
4	August 12	14.66	14.83	16.62	18.35
5	August 23	14.95	14.99	16.88	18.65
6	September 6	15.29	15.62	17.08	19.22
Per cent increase.............			2.1	11.7	25.9

TABLE 4

*Average weight per plant of stalks of asparagus cut during the season of 1920 and grades of stalks**

TREATMENT PER ACRE	YOUNG PLANTS					OLD PLANTS				
	Weight per plant	Increase over check	Grades			Weight per plant	Increase over check	Grades		
			Good	Fair	Poor			Good	Fair	Poor
lbs.	*gm.*	*per cent*				*gm.*	*per cent*			
Checks	149.7		72	33	28	478.7		158	60	55
150	146.3	−2.3	76	31	23	460.8	−3.7	130	63	77
300	157.5	5.3	75	33	29	490.0	2.4	141	64	89
500	175.4	17.1	81	25	45	535.4	11.7	181	79	109

* During the absence of the writer in the season of 1920 Prof. L. G. Schermerhorn of the Department of Horticulture took records on the counting and grading of the stalks. For this help the writer acknowledges his indebtedness.

Unfortunately, not all of the stalks were counted and graded throughout the season. The figures given are therefore only an indication of the direction in which the increase in weight seems to go.

A striking increase in weight per plant was obtained by the heaviest applications of salt, as compared with the plants from the check plots. The total weight of stalks produced by the old plants which received 500 pounds of salt per acre was 11.7 per cent higher than the total weight of stalks per plant produced on the check plots, and the total weight of stalks per plant of the

young plants grown on the plots receiving the same amount of salt per acre increased 17.1 per cent over the weight of stalks on the check plots. The total weight of stalks per plant from the plots receiving 300 pounds of salt was but slightly higher than the total weight produced on the check plots, both for old and for young plants, while the total weight of stalks per plant on the plots with applications of 150 pounds per acre was slightly below the total amount per plant on the check plots.

As may be seen from the available data for the cuttings during the first 24 days of the season, the increase in numbers was mainly in the poor grade. This is true both for the young plants and for the old plants. The number of poor stalks for the old plants on the check plots during this period was 20.1 per cent, while 29.5 per cent of the stalks grown on the plots which received 500 pounds of salt were of a poor grade. A similar grading and counting for the young stalks showed that 21 per cent of the stalks grown on the check plots, and 29.9 percent of the stalks from the plots which received the heaviest salt applications, were of a poor grade. These figures seem to indicate that much of the increase in weight can be found in an increase of poor-grade stalks; however, since this grading and counting is incomplete, no definite conclusion can be drawn. Besides, poor-grade stalks are usually thin, and their increase under the influence of the salt might be explained by the production of new stalks which, the following year, will produce a product of better grade.

The average weight per plant checks in general very closely with the countings of the stems and the height per plant of the previous summer. Keeping in mind the fact that the measurements and the cuttings represent only one year's work, it seems that the growth of asparagus is stimulated by salt. This conclusion seems to be justified also by the fact that the yield of the previous season was quite uniform. Earlier investigators accredited the beneficial effects of salt mainly to its ability to prevent the growth of weeds. The applications of salt in this case were entirely insufficient for such a purpose, for weeds grew as fast on the treated plots as on the untreated plots. From our work in the eradication of weeds by means of salt, it is known to us that far more than 500 pounds per acre is needed to prevent the growth of weeds. Besides, all plots with and without salt were kept fairly clean while measurements were taken. It appears therefore that there is a beneficial action of the salt itself on the growth and yield of asparagus grown on this particular soil. Asparagus can stand an extraordinary amount of salt, as is recorded elsewhere (8).

SUMMARY

Experiments with a top-dressing of sodium chloride, in addition to manure, were made upon 2-year-old and 11-year-old asparagus plants. Applications of common rock salt (Retsof agricultural salt) were made at the rate of 150, 300 and 500 pounds per acre. Cuttings were made the same season. Throughout the growing season following this cutting the stems of the plants were counted and the length measured at intervals of from 7 to 10 days.

The following spring the same amounts of salt were again applied, and the stalkscut. The stalks were weighed, counted, and graded for market purposes.

The number of stems and the total length of plants increased regularly in all series, the stems reaching their maximum height in the middle of the growing season.

At the last measurement taken, the average total lengths per plant of the 2-year-old asparagus plants on the plots receiving 150, 300 and 500 pounds of salt per acre were, respectively, 21.1, 28.6, and 38.7 per cent greater than the average lengths of plants on the check plots.

The counting of the stems was continued until September 6 and the increase in the average number of stems for the plots receiving 150, 300 and 500 pounds of salt per acre was found to be 2.9, 16.2 and 26.8 per cent, as compared with the check plots.

The average number of stems per plant on the 11-year-old plants increased 2.1, 11.7 and 25.9 per cent for applications of 150, 300 and 500 pounds of salt per acre, over the check plots.

The average weight of stalks per plant obtained the following year was increased for the young plants grown on plots receiving 300 and 500 pounds of salt per acre, by 5.3 and 17.1 per cent, respectively. For the old plants an increase of 2.4 and 11.7 per cent was observed. The production of the plants which received applications of 150 pounds of salt per acre decreased 2.3 per cent for the young plants and 3.7 per cent for the old plants, as compared with the average weight per plant on the check plots.

The increase in the number of stalks produced, and consequently in weight, seems to lie in the direction of the poorer grades. Emphasis is laid on the fact that the work represents only 2 years of cutting and 1 year of counting and measuring.

Asparagus grown on this soil seemed to be stimulated directly by the salt applications, for weeds were kept down by hand on all plots throughout the growing season.

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EXPERIMENTS WITH COMMON ROCK SALT: II. ERADICATION OF WEEDS AND CLEANING OF ROADSIDES WITH SALT[1]

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INTRODUCTION

The cleaning of roadsides and the eradication of weeds, with the progress of intense cultivation, becomes a problem which attracts more and more attention. Many attempts have been made to supplement the mechanical means of eradication of weeds and light underbrush by more rapid chemical methods. Salt is one of these means which can be used in reducing weeds and brush. Experiments reported by a number of investigators agree on the effectiveness of salt for killing weeds, but usually do not agree on the manner in which this effect is brought about. Cox (4) states that for eradication of ferns salt is a better spray material than either arsenite of soda or iron sulfate. With ferns at an average degree of thickness on the land, 150 pounds of salt dissolved in 60 gallons or more of water to the acre is sufficient. Two sprayings a year are recommended. Cox (3) also states that 125 pounds of common salt in 50 gallons of water will control such weeds as dandelion, mustard and blueweed. Jones and Orton (8) experimented with salt on knot-grass (*Polygonum aviculare*) and some of the common grasses (*Panicum*). They obtained satisfactory results with 640 to 3200 pounds of salt per acre used in dry form. Jones (7) found salt to be "the best chemical yet tried for killing the orange hawkweed, and when properly used it is entirely effective. Salt should be applied on a hot sunny day, preferably during a period of dry weather, and broadcasted in amounts of from 1 to 1.5 tons per acre." Blackman (1) experimented for 40 years with the salting of Canada thistles. He found that applications of salt while the thistles are moist with dew or rain will kill both tops and roots in pasture and cultivated land. Field experiments at Harper Adams Agricultural College (5), however, indicate that thistles do not seem to respond to treatment with salt, as the effect of judicious cutting is not intensified. At the Kansas Agricultural Experimental Station (6) applications of from 3 to 20 tons of salt per acre were made on areas infested with bindweed. Ten tons per acre was deemed sufficient to kill the weed, provided the small spots where the weed grows up were resalted. Russel (9)

[1] Paper No. 40 of the Journal Series, New Jersey Agricultural Experiment Stations, Department of Soil Chemistry and Bacteriology.

found that a solution of 125 pounds of salt in 50 gallons of water was effective for the eradication of mustard. Brenchley (2) states that salt is good on grass-land; 500 to 600 pounds per acre applied in the spring at the time nettles are cut down will do much to prevent this weed from springing up again.

EXPERIMENTS IN 1919

The experiments reported below were conducted during the years of 1919 and 1920 on the College Farm at New Brunswick and along the roadsides near Riverton, New Jersey, in an area infested by the Japanese beetle. The weeds included pigweed (*Chenopodium album*), orach (*atriplex pâtula*), ragweed (*Ambrosia trifida*), asparagus, dandelion, wild strawberry, hawkweed (*Hieracium aurantiacum*), knotgrass (*Polygonum aviculare*), ferns, and some of the common grasses and weeds which were not predominating. The shrubbery included poison ivy (*Rhus toxicodéndron* L.), five-leaved ivy (*Rhus radicans*), sassafras, oak, wild cherry, grape vine, hackberry, blackberry, alder, raspberry, and ash.

The soil on the College Farm consists of Sassafras loam, while the soil at Riverton and Hartford is a light sandy loam.

Applications of the salt were made broadcast at New Brunswick and at Riverton, on the weeds and on and around the stumps of the shrubs. At Hartford the salt was applied broadcast on all plots, no effort being made to treat the stumps separately. The brush on the plots at Riverton had been cut for some time and burned on the place before the salt applications were made. No cutting was done at New Brunswick. The salt applications varied from 2 to 8 tons per acre, and the size of the plots varied from 650 to 1250 square feet.

The rainfall during the year 1919 at New Brunswick was 52.41 inches, while the precipitation during the months of the experiments was 5.31, 8.33, and 6.30 inches for June, July, and August, respectively. The treatment of the plots, the date of application, the meteorological conditions about the time of application and the kind and condition of the growth on the weed and shrub plots at New Brunswick on July 12, and August 29, 1919, are given in table 1.

Although much of the ground vegetation was killed by the smaller applications weeds survived and sprang up again in the late fall. Where the heavy applications were made, 90 per cent of the weeds were killed, the poison and five-leaved ivies were injured and partly killed, but pigweed was not affected at all.

Experiments with the object of killing poison ivy were made during 1919, and the after-effects of the salt on these plots in 1920 are recorded in table 2. No other vegetation than poison ivy was found on these plots, the ground being thickly covered with sprouts from 1 to 3 feet high sent out by the vines which had rooted at every notch. These branches were not cut before the salt dressing was made. The salt was applied broadcast.

TABLE 1

Condition of weeds and shrubs on salt-treated plots during 1919, on Sassafras loam soil

PLOT NUMBER	TREATMENT PER ACRE	DATE OF APPLICATION	METEOROLOGICAL CONDITIONS	KIND OF GROWTH	CONDITIONS JULY 12, 1919	CONDITIONS AUGUST 29, 1919
	tons					
1	2.0	June 13	Warm, sunny day after several days of dry weather; 2 days later a shower	Pigweed, poison ivy, five-leaved ivy, (3–5 feet) hackberry, ground vegetation thick grasses, wild strawberry	Ground vegetation affected, some weeds dead	Generally not much affected; 40 per cent of weeds killed
2	2.5	June 13	Same	Same	About the same as no. 1	About the same as no. 1; about 50 to 60 per cent of weeds killed
4	3.0	June 13	Same	Same	Ground vegetation strongly affected	Most weeds injured or killed; poison ivy injured
6	3.5	June 13	Same	Same	About the same as no. 4, more weeds dead	About the same as no. 4
8	4.5	June 13	Same	Same	Strongly affected, most weeds dead, hackberry seriously injured; small poison ivy dead; five-leaved ivy little affected	Ground vegetation mostly killed; larger poison ivy but slightly injured, making healthy new shoots; pig weed growing
10	5.0	June 13	Same	Same	About the same as no. 8, but five-leaved ivy more affected	About the same as no. 8, but poison ivy had not as many new shoots; pigweed growing; other weeds 90 per cent killed
3, 5, 7, 9	Checks		Same	Same	On all plots vigorous growth	All plots thickly covered by weeds, etc.

TABLE 2

Condition of salt-treated poison ivy plots during 1919 and 1920 (Sassafras loam)

PLOT NUMBER	TREATMENT PER ACRE	DATE OF APPLICATION	METEOROLOGICAL CONDITIONS	CONDITION JULY 12, 1919	CONDITION AUGUST 29, 1919	CONDITION OCTOBER 8, 1920
	tons					
1	3	6/13	Warm, sunny weather after several dry days; 2 days later a shower	Little effect	40 per cent injured	No effect to be seen
2	4	6/13	Same	Small plants affected	20 to 30 per cent killed; about 30 per cent injured	Partly killed; larger plants (only slightly injured) making healthy new shoots
4	6	6/13	Same	60 per cent killed or injured	Approximately 50 per cent killed; larger plants made great number of new shoots	About 60 per cent less growth as compared with check plot
6	8	6/13	Same	Mostly killed or injured	80 to 90 per cent killed; remainder badly injured; very few new tiny shoots	Very little growth
3–5	Checks	6/13	Same	Vigorous growth	Vigorous growth	Plots thickly covered

TABLE 3

Condition of weeds and shrubs on salt-treated plots near Riverton, N. J., during 1919 on a light sandy loam soil

PLOT NUMBER	TREATMENT PER ACRE	DATE OF APPLICATION	METEOROLOGICAL CONDITIONS	KIND OF GROWTH	CONDITION AUGUST 11, 1919	CONDITION SEPTEMBER 4, 1919
	tons					
1	3	6/28	Dry weather, night following rain	Sassafras, alderberry, oak, vine, pigweed, raspberry, fern	Not very effective, some small stumps killed*	Some stumps killed; fern, vine, pigweed growing*
2	4	6/28	Same	Same	Great number of small stumps killed; sassafras alive; ferns killed	Stumps nearly all killed except few sassafras; weeds growing again, probably reseeding; ferns killed
3	4	6/28	Same	Sassafras, alder, oak, vine, pigweed, poison ivy, five-leaved ivy, orach	Number of small stumps killed; vine, poison ivy, pigweed slightly affected	Many stumps killed; pigweed and orache growing vigorously; 30–40 per cent of ivy killed
4	5	6/28	Same	Same	More effective than no. 3; poison ivy started up again; vigorous growth of orach	Larger stumps all killed; some small stumps apparently not touched by the salt were growing; few stumps made slight growth but leaves had sickly appearance, curled, small and brown; pigweed and orach growing vigorously
5	3	6/28	Same	Pigweed, vine, blackberry, tall grasses	Most of grass and blackberry killed	Blackberry and grass killed; vine little affected, pigweed seemed stimulated

* Notes on all plots are made in comparison with check plots.

Poison ivy can stand very heavy applications of salt. Even with 8 tons of salt per acre, not all of the stems were killed. The injury to the plants on this plot was severe, however. In October, 1920, but little growth was on the ground, while the check plots were thickly covered. If farmers want to rid their roadsides around their houses and fields, salt seems to be an excellent killer. To prevent growth entirely a resalting should be made on plants which have survived the first application. The second application need not be so heavy as the first.

The experiments at Riverton were conducted on another soil type, and included more brush and ferns. All growth was cut and the brush burned before the salt applications were made. The total rainfall for the months of June, July, August and September was 18.83 inches at Philadelphia (about 10 miles away).

An application of 5 tons of salt per acre killed nearly all stumps of sassafras, alder and oak, while poison ivy and five-leaved ivy were only injured. Pigweed and orach were not affected but grew vigorously. These weeds seemed to be stimulated by the smaller applications. An application of 3 tons of salt per acre killed the hardy grasses and the blackberry, while grape vines were but little affected.

EXPERIMENTS IN 1920

Experiments near Hartford, N. J.,[2] were conducted during the months of May, June, July, August and September, 1920. Here an effort was made to determine not only the amounts of salt needed to kill the weeds and brush, but also the time of the year at which the best results can be obtained, the influence of cutting weeds and brush before or after salt applications in comparison with uncut growth with salt applications, and the kind of salt which is best for weed eradication.

The different kinds of salt included Kerr's agricultural salt, Retsof agricultural salt, and a fine table salt sent by the United States War Department. Kerr's agricultural salt is a rather coarse salt. The Retsof agricultural salt is coarse and has a high percentage of sodium chloride, analyses running from 96 to 98 per cent NaCl. The salt from the War Department was a common fine table salt, not so well adapted to the work as Kerr's and Retsof agricultural salt, as it was hard and lumpy. The lumps could be broken only with difficulty, and remained for a long time on the plots without being dissolved.

Growth on the plots included ragweed, pigweed, wild strawberry, asparagus, hardy grasses, poison ivy, five-leaved ivy, wild cherry, hackberry, sassafras, sumac, wild grape and raspberry. Some of the wild cherry and sassafras were 4 to 6 inches in diameter, while other growth was very dense. When the vegetation was cut, as indicated in the table, the cutting was done immediately after the salt was applied. Work was begun May 1, and was continued

[2] The writer is indebted to John J. Davis and C. H. Hadley, entomologists in charge of the Japanese beetle control work at Riverton, New Jersey, for supplying these data, and for their cordial help in carrying out the other experiments at Riverton.

throughout the summer, the last application being made September 13. In all experiments, rain followed the applications of the salt within a short time and probably no particular application received an appreciable advantage over the others. The rainfall at Philadelphia during the months April to October was 27.72 inches.

The plots were frequently examined and the condition of plots 1 to 23, inclusive, on September 13 is recorded in table 4. The plots were examined again on November 12, and the notes taken on plots 24 to 30 are included in the same table. No difference from the examination on September 13 was noticed on plots 1 to 23.

Applications of 3, 4 and 5 tons of salt per acre killed most of the ground vegetation, as shown by a comparison with the check plots. Some of the stumps of wild cherry, sassafras, and hackberry were killed, but most of these were thrifty; pigweed, ragweed, poison ivy, five-leaved ivy and asparagus were thrifty. Such applications are insufficient to kill deep-rooted plants, such as poison and five-leaved ivies, sassafras, wild grape, and wild cherry, but the effects can be seen on these and on other plants enough to show appreciably between treated and untreated plots. As a rule 5 to 6 tons of salt per acre are effective in killing noxious plants if conditions are favorable.

Salt applications of 8 tons per acre were very effective in killing. Occasionally an unusally thrifty grape, sassafras or wild cherry survived. The shoots of these plants had made but very slight growth when the plots were examined again on November 12. It was very remarkable that asparagus plants survived (after cutting) on all plots. Even the heaviest applications did not do much harm to these plants, and applications of 4 tons per acre seemed to stimulate growth.

The deep-rooted plants were killed with difficulty; especially grape, five-leaved ivy, poison ivy, wild cherry, hackberry, sumac and sassafras, in the order named. Pigweed and ragweed were apparently unaffected by the salt applications; applications of as much as 8 tons of salt to the acre did little injury to these plants. Sometimes they even seemed stimulated by the salt and made greater growth on the salted plots than on the check plots. It is possible that NaCl affects this soil in such a way that it is more favorable for the growth of these weeds; or it may be that, with competition for space eliminated, these weeds have a better chance. At any rate, their growth shows that their vital activities are not harmed.

General observations on these experiments show that salt applications made early in the season are effective for brush and stumps, but less effective for weeds, probably on account of reseeding. Salting in the fall has very little effect. This was shown by earlier experiments conducted near Pleasantville, New Jersey, with uncut oak shrubs. Little or no effect could be seen during the following spring. Salt applications there did not exceed 4 tons per acre; these results are corroborated at Hartford where applications of 4 tons of salt per acre were given. The effects of salt applications after mid-season are very noticeable, but salting at about mid-season is most effective.

TABLE 4

Cut and uncut weeds and brush treated with different kinds of salt applied in different amounts and at different times of the year near Hartford, N. J.

PLOT NUMBER	DATE OF APPLICATION	CUT OR UNCUT	KIND OF SALT	TREATMENT PER ACRE	METEOROLOGICAL CONDITIONS	CONDITION SEPTEMBER 13, 1920
				tons		
1	5/1	Cut.	Kerr's agricultural	3.5	Rainy weather, temperature below normal	Pigweed, ragweed, poison ivy, asparagus, five-leaved ivy, wild cherry, all thrifty; hackberry, sassafras and sumac not very thrifty; hackberry leaves appear yellow spotted and deformed, resembling mosaic; grape foliage very small, scant, and unthrifty; apparently an appreciable number of stumps of wild cherry, sassafras, and hackberry did not come up; ground vegetation thin
2	5/1	Cut		Check	Same	Greater growth of sassafras and poison ivy; ground vegetation (grasses, ivy, etc.) noticeably heavier
3	5/11	Cut	Retsof agricultural	3.5	Rain in afternoon and following night	About the same as plot 1; sassafras probably less noticeably affected
4	5/11	Cut		Check	Same	About the same as plot 2
5	5/22	Cut	Same	3.5	Dry, sunny weather, rain 2 days later	About the same as plot 1
6	5/22	Cut	War department	3.5	Same	About the same as plot 1
7	5/22	Cut		Check	Same	Noticeably denser growth, not only the grass and ivy covering the ground but also a heavier growth of sassafras, wild cherry, etc.

8	6/10	Uncut	Retsof agricultural	4.0	Dry, sunny weather, next day shower	Two-thirds of sassafras stumps dead as well as many wild cherry stumps
9	6/10	Cut	Same	4.0	Same	No choice between this and plot 8
10	6/10	Uncut	War department	4.0	Same	About the same as plot 8; no apparent difference between plot 9 and this plot
11	6/10	Cut	Same	4.0	Same	Same as plot 10
12	6/10	Uncut		Check	Same	Ground vegetation vigorous; abundant growth of sassafras and poison ivy
13	7/7	Cut	Retsof agricultural	8.0	Heavy shower in evening	This plot showed best effects of all 1920 plots; absolutely everything dead except an occasional unthrifty grape or hackberry and a thrifty asparagus; all poison ivy killed
14	7/7	Uncut	Same	8.0	Same	Killed out completely raspberry, grass and all vegetation excepting wild cherry, grape and sassafras and these appreciably affected; all poison ivy and five-leaved ivy dead
15	7/7	Cut	Same	4.0	Same	Much of undergrowth, sassafras and vine killed; prickly ash (not occurring in above plots) less affected; apparently this treatment more effective than earlier plots where equal amounts of salt were applied; some wild cherry, sassafras and hackberry alive; most of poison ivy killed

TABLE 4—*Concluded*

PLOT NUMBER	DATE OF APPLICATION	CUT OR UNCUT	KIND OF SALT	TREATMENT PER ACRE	METEOROLOGICAL CONDITIONS	CONDITION SEPTEMBER 13, 1920
				tons		
16	7/7	Uncut	Same	4.0	Same	Not appreciably different from plot 15, except some growth of brush which might be expected as vegetation was uncut
17	7/7	Uncut		Check	Same	Heavy growth of vegetation; poison ivy abundant
18	8/5	Cut	Same	8.0	Dry weather, rained hard night following application	Not as complete a kill as in plot 13 but a fairly effective kill of vegetation; some poison ivy, five-leaved ivy, sumac and sassafras coming through; majority of vegetation killed off
19	8/5	Uncut	Same	8.0	Same	Not appreciably different from plot 18 except vegetation necessarily higher
20	8/5	Cut	Same	4.0	Same	Fairly effective in killing vegetation; noticeably not as effective as plot 13
21	8/5	Uncut	Same	4.0	Same	Not very effective
22	8/5	Cut		Check	Same	All growth started vigorously again
23	8/5	Uncut		Check	Same	Heavy growth of vegetation
						CONDITION NOVEMBER 12, 1920
24	10/13	Uncut	Retsof agricultural	4.0	Dry, sunny weather several days before rain	Not very effective; ground vegetation killed; some sassafras, sumac and hackberry injured, some killed
25	10/13	Cut	Same	4.0	Same	More effective than on plot 24, but apparently treated too late in season

26	10/13	Uncut		Check	Same	Vegetation thick
27	10/13	Uncut	Same	8.0	Same	Nearly everything killed, except some sassafras and poison ivy; much sassafras and poison ivy on this and plot 28
28	10/13	Cut	Same	8.0	Same	About same as plot 27
29	10/13	Uncut		Check	Same	Same as plot 26 but more sassafras and poison ivy
30	10/13	Cut		Check	Same	Stumps had made sprouts again

The plots on which the vegetation was cut just before or immediately after the applications of salt showed, in all cases, much better results than plots on which the high and rank vegetation was allowed to remain. There is, therefore no doubt about the effect of cutting when salt applications are made.

Some writers recommend applications of the salt in dry form, while others say that it should be applied in solution to procure the best results. The reason why investigators differ as to the effect of the salt as a weed-killer seems to lie in the fact that the action of NaCl upon the plants is not well understood. True (11) states that salt has slight toxic effects upon some plants but that most of its value as a weed-killer depends upon the fact that it rapidly draws the water out of the plant tissues with which it comes in contact. This view is still held by many workers. All of our experiments indicate that the injury is caused by the chlorine content taken up by the plant; at least far more than by the absorbing of water out of the plant tissues. Besides, the latter could be true only for weeds with broad, outstanding leaves, such that salt could remain on them for a considerable length of time. But the leaves of grasses are long, narrow, and upright, so that even moisture does not adhere to them for a long time. Cut or burnt weeds and stumps have but few, if any, leaves left, and dry salt could not be effective. The toxicity of chlorine is well known. In our experiments the best results were obtained when the salt was brought into solution by rain soon after the applications had been made; the salt, being dissociated, could then be taken up by the plants. Laboratory experiments showed that equivalent amounts of salt added to a sandy soil with 30 per cent of the optimum moisture content were not so effective as the salt applied to a sandy soil with an optimum moisture content, or 60 per cent of the water-holding capacity of the soil.

The flocculation of a soil is more pronounced when there is an excess of NaCl; thus settlement of the fine soil particles is more rapid. When NaCl is applied to pots, an impermeable crust is formed which, with the change in the structure of the soil, results in interference with the ordinary aeration of the soil. Freezing-point determinations of the salted soil extract showed in laboratory experiments that the osmotic concentration of the soil moisture became greater, preventing the roots of the plants from taking up unlimited amounts of water. If, therefore, lack of water was the cause of injury, the salt in the pots with 30 per cent of the optimum moisture content should be more active.

Great quantities of NaCl applied to a soil decrease plant growth on account of the toxic effects, but when washed away or leached out sufficiently, salt stimulates growth. When added in small quantities to asparagus it stimulates directly. It does not seem plausible that this stimulating effect is due to the sodium content, for in the experiments with asparagus, sufficient potassium seemed to be available. Since the experiments of Schulze (10) have shown quite conclusively that the effect of NaCl on liberating potassium is

not universally important, it seems safe to conclude that the Cl ion taken up in small quantities by the plant stimulates growth; but when absorbed in larger quantities, causes injury.

SUMMARY AND CONCLUSIONS

Experiments with sodium chloride for killing weeds and brush were conducted along roadsides on two different soil types in 1919 and 1920. Applications of from 2 to 8 tons of salt per acre were made.

At New Brunswick and Hartford the salt was broadcasted, while at Riverton the stumps of shrubbery were treated more severely.

The objects of the experiments were: to determine the necessary amounts of salt needed to kill weeds and brush; to learn at what time of the year best results can be obtained; to learn whether or not the cutting of weeds and brush before or after salt applications has influence on the effects; and to determine which kind of salt (fine or coarse) is best for these purposes. The different kinds of salt used were Kerr's agricultural salt, Retsof agricultural salt and ordinary table salt.

Plant growth observed in these experiments included pigweed, orach, ragweed, asparagus, dandelion, wild strawberry, hawkweed, knotgrass, ferns, hardy grasses, poison ivy, five-leaved ivy, sassafras, oak, wild cherry, wild grape, hackberry, blackberry, alder, raspberry, and ash.

The following tentative conclusions may be drawn:

1. Poison ivy is very difficult to kill, but 8 tons of common rock salt per acre will kill this plant and prevent growth the second year, provided smaller applications are made to the plants which survive the first application.

2. Two and 2½ tons of salt per acre kill the ground vegetation and many weeds, but the effect does not last long enough to give good results.

3. Three to four tons of salt per acre is not sufficient to kill all deep-rooted plants, such as poison ivy, five-leaved ivy, sassafras, wild grape, and wild cherry; but these amounts do affect these and other plants and check growth sufficiently to show an appreciable difference between treated and untreated plots.

4. As a rule, 5 to 6 tons per acre is effective if conditions are favorable; that is, when applications are made on a rainy day or when rain occurs within a few days, and when all vegetation is cut.

5. Eight tons of agricultural salt will kill all vegetation except asparagus.

6. Salting is most effective about mid-season, or shortly thereafter.

7. High and rank vegetation should be cut immediately before or after applications.

8. Table salt dissolves less rapidly and is less effective than ground rock salt, commonly known as agricultural salt.

9. Deep-rooted plants are very difficult to kill, especially grape, five-leaved ivy, poison ivy, wild cherry, hackberry, sumac, and sassafras, in the order

named. Pigweed and ragweed are not much affected by the salt. Asparagus is apparently unaffected even by heavy applications.

10. It is thought that the chlorine ion, taken up in small quantities by the plants tested, stimulated growth; but when absorbed in large quantities caused injury and death.

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EXPERIMENTS WITH COMMON ROCK SALT: III. AFTER-EFFECTS OF SALT[1]

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Salt is one of the most effective herbicides and one of the most useful materials for sterilizing the soil. But those who use salt as a means for killing undesirable vegetation along drive-ways and railroads, on tennis courts, and the like, have often noticed that, while a heavy application of salt subdues the vegetation for a period of from 18 to 24 months, as soon as the excess of salt goes out of the soil, the residue apparently acts as a fertilizer; and one or two years after the treatment the vegetation on the treated places is frequently more vigorous than that on untreated areas alongside.

The after-effects of salt on alfalfa, rye grass, rye, silver beet, wheat, rape, Canada grass, and field pea have been studied by Ewart (1). He made applications of from 200 to 6400 pounds of salt per acre, duplicating each time the amounts used on the foregoing plots. In the second year after the treatment insufficient salt remained to injure the crops directly, and a decided increase occurred in most of the crops tested.

The experiments on weed eradication conducted in 1919 and reported in another paper (4) were used in 1920 to determine the after-effects of the salt. Plots treated with 3 tons of salt per acre showed no decided difference in weed growth from the check plots in October, 1920, except that pigweed seemed more abundant. Plots which received applications of 3.5 and 4 tons per acre had fewer weeds than the check plots, and the number of weeds on the plots which had received 5 tons of salt were not more than 40 to 50 per cent of those on the check plots, while some species of weeds did not reappear at all. The growth of poison ivy on these plots was decidedly less than in 1919, as could be seen by a general counting of sprouts. If applications are made heavy enough, weed growth seems to be prevented for a period longer than two growing seasons. On the other hand, when but 2 and 2.5 tons of salt per acre were applied, weed growth seemed far more abundant than that on the check plots at the end of the second growing season.

Experiments made in the spring of 1919 (2) in a cut-over swamp near the College Farm at New Brunswick, New Jersey, with a number of selected tree

[1] Paper No. 41 of the Journal Series, New Jersey Agricultural Experiment Stations, Department of Soil Chemistry and Bacteriology.

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TABLE 1

Condition of tree stumps 4 months and 18 months after treatment with common rock salt

STUMP NUMBER	VARIETY	SALT APPLIED	CONDITION AUGUST 7, 1919	CONDITION OCTOBER 9, 1919
		lbs.		
1	Birch	1	Healthy appearance	Growth of new shoots good
2	Oak	1	Healthy appearance	Growth of new shoots good
4	Maple	1	Healthy appearance	Growth of new shoots good
10	Dogwood	1	Healthy	Good new shoots
11	Dogwood	2	Healthy	Good new shoots, 3 feet
14	Chestnut	2	Injured	New shoots, very vigorous, 6 to 7 feet
17	Birch	2	Vigorous growth	Very good new shoots
19	Maple	2	Seriously injured	Alive
20	Oak	2	Healthy	Extremely vigorous new shoots, 6 feet
22	Oak	3	Bluish colored, large leaves	Healthy but few new shoots, 4 feet
24	Chestnut	3	Injured	Dead, with few poor new shoots
26	Birch	3	Slightly injured	Good new shoots
27	Birch	3	Healthy	Very vigorous new shoots, 6 feet
28	Cherry	3	Healthy	Vigorous new shoots, 5 feet
33	Oak	4	Injured	Dead
34	Cherry	4	Injured	Stem dead; good new shoots, 4 feet
36	Oak	4	Main stem healthy, side stems injured	Vigorous shoots with large leaves, 5 feet
40	Birch	4	Sickly appearance	Poor new shoots, 2 feet
43	Birch	5	Top shoots dead	Very good new shoots, 4 feet
48	Maple	5	Sickly appearance	Dead
49	Oak	5	Healthy	Very good new shoots, 4 feet
52	Birch	6	Dead	Dead
54	Sumac	6	Sickly appearance	Very poor new shoots, 1 foot
55	Oak	6	Healthy	Good new shoots with large leaves, 3 feet
59	Maple	6	Dead	Dead
60	Oak	6	Healthy with bluish leaves	Poor shoots with shiny leaves, 2 feet
62	Oak	7	Injured	Very good new shoots, large leaves, 4 feet
65	Oak	7	Healthy	Poor shoots, 2 feet
66	Birch	7	Dead	Dead
69	Maple	7	Injured	Poor new shoots, 2 to 3 feet
71	Oak	8	Injured	Dead except two extremely poor shoots, $\frac{1}{2}$ to 1 foot
72	Birch	8	Dead	Dead
78	Oak	8	A number of leaves brown, dried	Extremely vigorous shoots, 7 feet
79	Maple	8	All leaves dropped	Dead
81	Oak	9	Slightly injured	Extremely vigorous shoots, 7 feet

TABLE 1—*Concluded*

STUMP NUMBER	VARIETY	SALT APPLIED	CONDITION AUGUST 7, 1919	CONDITION OCTOBER 9, 1919
		lbs.		
82	Chestnut	9	Seriously injured	Dead
84	Oak	9	Partly injured	Alive, no shoots
85	Oak	9	Injured	Dead, no shoots
89	Birch	9	Seriously injured	Dead
90	Oak	9	Partly injured	Very vigorous shoots, broad, shiny leaves, 6 feet
91	Oak	9	All veins on back of leaves yellowish	Extremely vigorous stems, 7 feet
94	Maple	10	All leaves that are left are brown and curled	Dead except two very poor shoots
95	Birch	10	Most of stems dead; small new shoots	Dead
97	Ash	10	Dead except two small new shoots	Dead except two very poor shoots
100	Oak	10	Sickly appearance	Dead
101–130	Oaks, maples, birches, etc.	None	All healthy	Fairly good shoots

stumps which were treated with common rock salt, gave an opportunity to make comparative notes in the fall of 1920 on the after-effects of the salt. Unfortunately, a fire ran through a part of the woods some time in the early spring of 1920, destroying much of the tall brush but not killing the roots. Growth soon started again. Of the one hundred treated tree stumps, a number of representative stumps were selected and their condition reported in table 1.

A study of these notes shows that when the stumps were not injured by the treatment a stimulating effect of the salt resulted. If the growth was but slightly affected by the salt applications, a vigorous growth of new shoots appeared. Not only were the injurious effects overcome, but healthy shoots of from 3 to 5 feet in height were produced. This was especially true of the hardy oak. If, however, the tree stumps had been seriously injured a very poor growth of new shoots was produced. In some cases death of the stumps resulted.

Different results were observed on the plots at Bridgeton, New Jersey (3), where oak stumps were treated with salt. These stumps were cut and burned at the place before the salt was applied. Examinations made on May 15, 1919,[2] showed that on all the plots which had received salt there was much less growth than on the check plots. On the check plots there was much undergrowth of blueberry which had been killed where the salt had been applied

[2] On account of the writer's absence, the examinations were made by Dr. E. Van Alstine, of the New Jersey Agricultural Experiment Station.

broadcast. Aside from killing the small undergrowth, salt applied broadcast showed no effect. The tree sprouts were as vigorous and as early as those on the checks. A difference could be noticed on plots 7, 8 and 9 which received 4200, 4750 and 5300 pounds of salt per acre, respectively, applied on and around the stumps. A number of stumps appeared to be dead, and all vegetation was smaller and less vigorous than that on the check plots, and was not so far advanced.

During the fall of 1920 these plots were examined again and they showed very much the same conditions as were observed at the time of the earlier examination. A counting of live stumps on the plots which had received 4750 and 5300 pounds of salt per acre showed that 60 to 70 per cent of the stumps were dead or had made such slight growth that they appeared to be still suffering from the treatment. The remainder of the stumps on these plots had a far less vigorous growth than those on the check plots.

The burning over of the stumps before treatment seemed to be of great value in this effort to kill live stumps with salt. No stimulating effect could be noticed on the plots which received the smaller amounts of salt. On the contrary, all growth appeared to be less vigorous.

CONCLUSIONS

The injurious action of heavy applications of 4 and 5 tons of salt per acre was still perceptible in the second year for weeds and brush, but was practically imperceptible where medium applications of 3 and 3.5 tons of salt per acre had been made. Where small applications of 2 and 2.5 tons had been made, a fertilizing action seemed to take place.

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THE OXIDATION OF SULFUR BY SOIL MICROÖRGANISMS: I[1]

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INTRODUCTION

It is now well established that sulfur, when added to the soil, is oxidized to sulfuric acid. This can be demonstrated by the increase both of the acidity of the soil and the amount of soluble sulfates. This knowledge has been utilized in converting insoluble into soluble phosphates so as to make them more readily available for plant growth, without recourse to the use of commercial sulfuric acid, for the manufacture of superphosphates. Extensive investigations of this problem have been carried on at the New Jersey Agricultural Experiment Station (6, 8). They have established certain important facts relating to sulfur oxidation, and the formation of soluble phosphates from ground rock phosphate. A detailed review of the earlier work on sulfur oxidation in soil is given in the above mentioned publications.

A few of the results obtained that have an important bearing on the work at hand, may be summarized as follows:

1. The oxidation of sulfur in sand and soils was found to render soluble in water and in neutral ammonium citrate appreciable quantities of the phosphorus of the pulverized phosphate.
2. The oxidation of sulfur as measured in terms of sulfates paralleled the increase of available phosphoric acid.
3. The oxidation of sulfur and the rendering available of the phosphorus in rock phosphate is influenced by a biological factor. In inoculated composts (soil, sulfur and rock phosphate), all other conditions being the same, the quantity of phosphorus rendered available in 9 weeks was double that made available in uninoculated composts in 30 weeks.
4. The microorganisms which oxidize the sulfur appear to be aerobic.
5. Calcium carbonate may bring about a decrease in available phosphorus when added to sulfur-floats-soil composts.
6. Composts are more efficient in producing available phosphates in the absence of large amounts of organic materials, such as peat, fresh horse manure, old composted manure and peptone. The more soluble organic materials will bring about the greatest decrease in efficiency.
7. Sulfur-oxidizing microorganisms may develop more rapidly in the absence of soluble organic matter. They appear to be similar in this respect to the nitrifying organisms.
8. When peptone was added to a mixture of soil, sulfur and floats, the sulfur was transformed largely into sulfites.

[1] Paper No. 42 of the Journal Series, New Jersey Agricultural Experiment Stations, Department of Soil Chemistry and Bacteriology.

These conclusions were based entirely upon the results obtained with crude cultures of bacteria in the composting of sulfur, floats (phosphate rock) and either soil, sand or manure. The present paper deals with an organism which oxidizes sulfur very rapidly, and which has been grown in pure culture for many generations. The methods used in the isolation of the organism (or one of the organisms) concerned in the oxidation of sulfur, are described, and an attempt is made to interpret the work of Lipman and McLean (8) and of McLean (6), in terms of the changes produced by pure culture of the sulfur-oxidizing bacteria.

THE ISOLATION OF THE SULFUR-OXIDIZING ORGANISM

A fairly comprehensive bibliography on sulfur-oxidizing organisms is found in a recent paper by Düggeli (3). It may be noted in this connection that in most of the work on the oxidation of sulfur by microorganisms, the starting point was not elementary sulfur, but hydrogen sulfide, sulfides or thiosulfates. The oxidation of sulfur was measured, in most cases, not by the production of sulfuric acid and sulfates, but either by the disappearance of sulfide in the medium, or by the appearance or disappearance of sulfur granules within or without the microbial cell. Then, we must point out the fact that most of the earlier investigations have dealt with organisms isolated from sulfur springs, salt and fresh waters and curative muds in Russia, and only a few investigations were concerned with sulfur-oxidizing organisms in the soil. Of the latter, the most important are those of Beijerinck (1) and Jacobsen (4, 5) who have described two sulfur-oxidizing organisms, *Thiobacillus denitrificans and Thiobacillus thioparus* which oxidize sulfides, thiosulfates and elementary sulfur. A comparison of these organisms with the one ioslated by the writers is published elsewhere (10).

The oxidation of sulfur in the composts (sulfur-rock-phosphate mixture) is always accompanied by an intense acid production, even in the presence of a neutralizing agent, in this case, the tri-calcium phosphate. We may cite here a table from a paper by Lipman and Joffe (7):

Change of pH value in the oxidation of sulfur in sulfur-rock-phosphate composts

	TIME OF INCUBATION													
	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks	8 weeks	9 weeks	10 weeks	12 weeks	14 weeks	16 weeks	18 weeks	20 weeks
pH	4.4	3.0	2.8	2.8	2.6	2.4	2.6	2.4	2.3	2.2	2.3	2.2	2.0	2.0
Available P, (per cent)	3.75	4.38	14.90	15.44	19.11	27.2	33.4	34.9	44.2	59.9	61.0	66.6	77.6	85.1

The acidity shows a rapid gain, then a slow variable change, then a rapid increase again, but much smaller than the first.

It is characteristic that the phosphate becomes soluble only after a definite period of composting which results in a rise in acidity as expressed in terms of hydrogen-ion concentration. Hence, the organism or organisms concerned in the oxidation of the sulfur would be expected, when isolated in pure culture, to produce an intense acidity in the medium.

Much preliminary work was carried out before the proper medium and methods were developed for the isolation of the organisms. Only such of the experiments will be reported here as have led to the direct isolation of the organisms concerned.

PRELIMINARY MEDIA USED

Medium A

	gm.
$(NH_4)_2SO_4$	2.00
K_2HPO_4	1.00
$MgSO_4$	0.50
KCl	0.50
$FeSO_4$	0.01
Dextrose	10.00
Sulfur	10.00
$Ca_3(PO_4)_2$	10.00
Distilled water	1000.00

Medium B

Same as A, but 1 gm. of dextrose was used in place of 10 gm.

The hydrogen-ion concentrations of these media, after sterilization, was pH = 6.0 to 6.2. All the constituents, with the exception of the tri-calcium phosphate and sulfur, were first dissolved in water; 100-cc. portions of the medium were then distributed in 250-cc. Erlenmyer flasks, the tri-calcium phosphate and sulfur were weighed out separately, and 1-gm. portions of each were added to each of the flasks. The flasks were then plugged with cotton and sterilized in flowing steam for 30 minutes on three consecutive days. The flasks and contents were always incubated, after inoculation, at 26° to 28°C.

METHODS OF ANALYSIS

At the end of a definite incubation period, the culture was filtered, and 17 cc. of the filtrate used for the following determinations:

1. The pH values were obtained from 2-cc. portions of the culture by the use of the colorimetric method, the phenolsulfonphthalein series of indicators being used, as outlined by Clark and Lubs (2).

For pH determinations of less than 1.2, the indicators mauvein and methyl-violet were used, as suggested by Sörensen (9), and checked by the electrometric method.

2. Titratible acidity was determined by titrating 5 cc. of the culture against 0.1N NaOH, with phenolpthalein as an indicator. The amount of the NaOH used per 1 cc. of the culture was recorded as titratible acidity (or titration).

3. Soluble sulfates were determined as follows: 5 cc. of the culture was neutralized with ammonia, acidified with 1 cc. of concentrated hydrochloric acid, diluted to 150 cc. and the sulfates precipitated, at the boiling point, with 10 cc. of $1.0N$ $BaCl_2$ diluted to 50 cc. From the weight of the $BaSO_4$ obtained the amount of soluble sulfates was calculated as SO_4 for 100 cc. of the original culture.

4. Insoluble sulfates were determined as follows: The residue from 100 cc. of culture in one flask, after filtering off the liquid, was treated with 10 cc. of concentrated hydrochloric acid and 150 cc. of distilled water, and the contents digested on a water bath for four to five hours. This brought into solution everything except the unoxidized residual sulfur. The liquid was decanted into the filtrate originally obtained (or residual part thereof); the undigested residue was then washed with distilled water until no test for sulfates could be obtained in the liquid. Occasionally, a second digestion for one or two hours with 5 cc. of hydrochloric acid was required. The filtrates were collected and made up to volume; an aliquot portion was taken and the sulfate determination was made according to the method described under paragraph 3. This gave the total sulfates present. If a part of the original filtered culture was used for the other determination, the soluble sulfates present in the 17 cc. used for those determinations were added. By subtracting the soluble sulfates from the amount of total sulfates, the insoluble sulfates were obtained.

5. The soluble phosphates were determined gravimetrically in a 5-cc. portion according to the methods of the Association of Official Agricultural Chemists. The percentage of soluble phosphates was calculated by dividing the difference between the soluble phosphates in the culture and the soluble phosphates in the control by the difference between the total phosphates and the soluble phosphates in the control.

6. The residual sulfur was found by washing into a Gooch crucible the residue from the hydrochloric acid digestion, obtained as described in paragraph 4. It was then dried in an electric oven at about 60°, until constant weight was obtained.

Procedure

Small samples were taken from composts, in which the oxidation of sulfur was well advanced, and brought into the laboratory. Several flasks of the two media were inoculated with 1-cc. suspensions made from the different composts. Different dilutions were used so as to free, if possible, the sulfur-oxidizing organisms from any contaminating organisms. The dilutions used were: 1:10, 1:1000, 1:100,000, and 1:10,000,000.

It may be seen in the table 1 that while good growth was obtained with the lower two dilutions in both media, oxidation of the sulfur, as expressed by the change in reaction, was observed only in medium B. But a slight change in reaction was observed in medium A.

Since the only difference between the two media was the dextrose content, one might assume that an excess of dextrose (1 per cent) would be injurious to the development of the sulfur-oxidizing organisms. The H_2S odor in the culture of A, with the lower dilution, would indicate reduction rather that oxidation phenomena. However, to get further light on the influence of dextrose upon the development of the organism in question, transfers were made from the cultures with the lower dilutions (1:10 and 1:1000) in A and B to fresh sterile flasks containing the same media as in the flasks from which the transfers were made.

To test further the action of this culture on medium I, a series of flasks were inoculated alike (by means of a platinum loop) and incubated.

TABLE 1

The growth of sulfur-oxidizing organisms in artificial culture media

	MEDIUM A				MEDIUM B			
	1:10	1:1000	1:100,000	1:10,000,000	1:10	1:1000	1:100,000	1:10,000,000
Growth*.......	3	4	Trace	None	4	3	2	None
Odor..........	H_2S	H_2S	None	None	Pungent	None	Faint H_2S	
pH at start....	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
pH at the end of 7 days......	5.9	5.5	6.0	6.0	3.0	3.8		
pH at the end of 14 days.....	5.4	5.5	6.0	6.0	2.8	3.3	4.4	6.0

*2 = poor, 3 = fair, 4 = good.

The influence of dextrose in preventing the rapid oxidation of the sulfur is revealed in table 2. In the presence of dextrose, the pH of the medium became, on transfer, 5.6, 5.2 and 4.9 after 7, 14 and 21 days, respectively, for the lower dilution, and 4.9, 3.8 and 4.0 for the higher dilutions, on the corresponding dates; with only 0.1 per cent of dextrose the pH became 2.0, 1.9 and 1.9 for the lower dilutions on the same dates. This was also accompanied by a greater accumulation of soluble sulfates and phosphates. The same relationship held true on successive transfers with the only difference that it took a longer period of time to bring down the pH value to the same point.

TABLE 2

Development of sulfur-oxidizing organisms in first transfer with the same culture medium

MEDIUM	DILUTION	INCUBATION 7 days	14 days	21 days	28 days	GROWTH	SOLUBLE PHOSPHATES (AS P) IN 100 CC. IN 28 DAYS	SOLUBLE SULFATES (AS SO_4) IN 100 CC. IN 28 DAYS
		pH	*pH*	*pH*	*pH*		*mgm.*	*mgm.*
Control..........		5.8	5.8	5.8	5.8	0	44.5	207.0
Medium A.......	1:10	5.6	5.2	4.9		4	40.9	
	1:1000	4.9	3.8	4.0		3		
Medium B.......	1:10	2.0	1.9	1.9	1.8	4	74.2	542.7
	1:1000	1.9	1.6	1.5	1.6	4	77.2	779.5

The complete elimination of dextrose from the medium led to a more rapid oxidation of sulfur, as shown by a greater acidity and larger amounts of soluble

and insoluble sulfates accompanied by larger amounts of soluble phosphates, indicating that a dextrose-free medium was more favorable for the development of the organism. This medium consisting of the pure minerals in amounts given above under A and B, was now adopted for constant work and called medium I. The only difference between this medium and the other two preliminary media, was the complete elimination of dextrose in the former. When 100-cc. portions of this sterile medium in 250-cc. Elenmeyer flasks were inoculated from the cultures grown in the third transfer on the previous medium containing 0.1 per cent of dextrose, the results shown in table 3 were obtained.

TABLE 3

The development of the sulfur-oxidizing organisms on dextrose-free medium (Medium I)

AGE OF CULTURE	pH	SOLUBLE PHOSPHATES (AS P) IN 100 CC.	SOLUBLE SULFATES (AS SO_4) IN 100 CC.	TITRATION
days		*mgm.*	*mgm.*	*cc.*
Control	6.0	44.5	185.1	0.16
7	1.8			
14	1.2			
21	1.3	77.3	962.5	2.09

The impure cultures of the sulfur-oxidizing organisms are thus found to possess very strong oxidizing properties, 188 mgm. of the sulfur having been oxidized in 14 days, and 260 mgm. in 28 days. The conversion of the tri-calcium insoluble salt into soluble di- and mono-calcium phosphates brought about an exhaustion of the medium in basic substances and, therefore, to a slower accumulation of acids, resulting from the oxidation of the sulfur. This explains the slower oxidation of sulfur after the initial period of rapid oxidation. Sulfuric acid produced from the oxidation of sulfur reacted with the insoluble tri-calcium phosphate and converted it into soluble forms. In 7 days, we already find nearly 116 mgm. of phosphorus, or 73.6 per cent of the original material, converted into soluble forms. The reason that not all the phosphate was made water-soluble is explained by the fact that the tri-calcium phosphate is converted into di- and mono-calcium phosphate, of which only the latter is completely water-soluble, while the former is only slightly soluble in water.[2]

Thus far the work had been carried out not with the pure, but with crude cultures of sulfur-oxidizing organisms. At first molds and bacteria would develop, particularly in the presence of dextrose and in the lower dilutions.

But with the higher dilutions and with the elimination of dextrose from the medium, the molds were eliminated and only a bacterium developed which did not form any pellicle on the surface of the medium but grew throughout the medium, making it uniformily turbid. The bacterium, a minute rod-shaped organism, was accompanied by a larger one, which did not occur uniformly in the different media and repeated transfers.

[2] The physico-chemical principles involved will be taken up in a later paper.

Attempts to cultivate the organisms on various organic and inorganic solid media failed. Media containing flowers of sulfur, reprecipitated sulfur, or sodium thiosulfate as a source of energy, in addition to the other minerals, and washed agar or silica jelly as a solidifying agent, did not produce any growth. To obtain pure cultures in the liquid media, the problem was attacked in three different ways:

1. By transferring the culture repeatedly into flasks with sterile medium (no. I) having uniform composition.
2. By using high dilutions of the cultures on transfer, so as to eliminate the contaminating organisms.
3. By starting with a medium of a high acidity.

It was expected that the organism which does not oxidize sulfur would probably not be able to develop at the very high acidity, even if a few cells were introduced into a fresh portion of the medium. By the use of all these three methods of procedures, we finally succeeded in eliminating the contaminating organisms, leaving the sulfur-oxidizing bacterium in pure culture.

The purity of the culture could be demonstrated only microscopically, since the organisms refused to grow so far on any of the solid media tried. The use of very high dilutions (1:100,000) in obtaining the culture and in several transfers, as well as the high initial acidity used in a few instances (up to a pH = 2.0) tended to eliminate the contaminating organisms and leave only the bacterium that can use elementary sulfur as the only source of energy, and CO_2 from the air as the only source of carbon. The purity also was proven physiologically (negative proof) by inoculating the organism upon various media commonly employed for microbiological work, without any growth taking place.

The organism was kept in culture on medium I and transferred about every 7 to 14 days. Although it continued to live in this medium, it seems that the high acidity exerted a deleterious action upon it, for on continued transfer, it took a longer period of time for the reaction (pH value) to reach a certain point than on previous transfers, thus indicating that the vitality of the organism was gradually diminishing on continued cultivation on artificial culture media. Also, in transferring, larger amounts of inoculum were required to produce a good growth. This may probably be due to the fact that a large number of the bacteria in the inoculum were dead cells, or had lost their vitality or ability to reproduce.

COURSE OF SULFUR OXIDATION BY THE SULFUR-OXIDIZING BACTERIUM

To follow in detail the process of sulfur oxidation, acid production and conversion of tri-calcium phosphate into soluble forms, a set of 60 flasks (250-cc. Erlenmeyer) each containing 100-cc. of medium I were prepared and sterilized. All the flasks were inoculated alike from the same culture and placed in the incubator. At the end of various intervals, 3 to 6 flasks were

taken at random from the set of 60 or remaining flasks and analyzed. The results are averaged and presented in table 5.

On studying the results presented in table 5, we observe some very important facts concerning the oxidation of sulfur, production of acid and transformation

TABLE 4

The oxidation of sulfur and the transformation of insoluble calcium phosphate into soluble phosphates by impure cultures of sulfur-oxidizing organisms

AGE OF CULTURE	pH	SOLUBLE SULFATES (AS SO_4) IN 100 CC.	INSOLUBLE SULFATES IN 100 CC.	TOTAL SULFUR OXIDIZED	PROPORTION OF THE TOTAL SULFUR ADDED THAT WAS OXIDIZED	SOLUBLE PHOSPHATES (AS P) IN 100 CC.*	INSOLUBLE PHOSPHATES MADE WATER-SOLUBLE
days		*mgm.*	*mgm.*	*mgm.*	*per cent*	*mgm.*	*per cent*
Control	6.0	185.1	0.0	0.0	0.00	44.5	20.4
7	2.0	503.9	0.0			160.3	73.6
14	1.8	352.0	354.0	188.0	18.80	147.2	67.5
21	1.6						
28	1.4	403.7	398.1	260.5	26.05	149.1	68.5

* Total phosphorus in 100 cc. of the medium (as P) = 217.9 mgm.

of tri-calcium phosphate into soluble forms. The results are quite irregular, in spite of the fact that each of these represent averages of three or more flasks. But in view of the fact that a detailed study of the metabolism and life history of this organism has not been made as yet, as well as the fact that

TABLE 5

Course of reaction of the sulfur-oxidizing organisms

AGE OF CULTURE	pH	TITRATION	SOLUBLE SULFATES (AS SO_4) IN 100 CC.	INSOLUBLE SULFATES (AS SO_4) IN 100 CC.	TOTAL SULFATES (AS SO_4), IN 100 CC.	PROPORTION OF THE TOTAL SULFUR ADDED THAT WAS OXIDIZED	SOLUBLE PHOSPHATES (AS P) IN 100 CC.*	INSOLUBLE PHOSPHATES MADE WATER-SOLUBLE
days		*cc.*	*mgm.*	*mgm.*	*mgm.*	*per cent*	*mgm.*	*per cent*
At start	5.4	0.16	209.78	0.00	209.78	0.00	45.57	0
1	5.4	0.15	207.77	0.00	207.77	0.00	42.61	−1.7
2	5.3	0.18	213.98	0.00	213.98	0.14	47.20	0.9
4	4.6	0.24	226.30	20.30	246.60	1.22	55.00	5.5
6	3.5	0.62						
8	2.6	0.94	468.03	159.39	627.42	13.92	103.56	33.7
10	2.7	0.85	336.61	159.75	496.36	9.55	92.97	27.5
12	2.5	0.90	241.54	307.68	549.22	11.32	186.30	81.7
15	2.4	1.00	269.69	295.45	565.14	11.85	207.28	93.9
19	2.3	1.09	288.56	278.97	567.53	11.93	194.20	86.3
23	2.4	1.09	300.22	262.97	563.19	11.78	193.45	85.9
30	1.7	1.73	486.75	351.40	838.15	20.95	193.85	86.1
38	1.8	1.60	288.64	377.52	666.16	15.21	(146.8)	(58.8)
68	1.7	1.95	376.58	343.31	719.89	17.00	193.57	85.9
120	0.8	6.80						

* Total phosphates in solution (as P) = 217.8 mgm.

we are in the dark as to the influence that the concentration of acid in the mother culture has upon the vitality of the organism, the influence of the age of the mother culture, amount of inoculum, etc., may have to do with the great variability of the individual flasks. Since investigations on these facts have not been brought as yet to any successful conclusion, we might attempt to consider the results presented in table 5 at their face value without attempting to make any sweeping conclusions.

The increase in acidity expressed in terms of both hydrogen-ion concentration and titratable acidity, is found to rise steadily with the increase in the period of incubation. The slight irregularities are due to the variability pointed out above. We find two important changes, one corresponding to the period between 4 and 8 days, when there is a rapid increase in acidity accompanied by a large accumulation of soluble and insoluble sulfates. We will note that the solution of the tri-calcium phosphate lags behind the increase in acidity and soluble sulfates: from the tourth to the eighth day there is a change in pH from 4.6 to 2.5 (2.5–2.7), and in titration from 0.24 to 0.94, while the soluble sulfates increased from 226.3 mgm. per 100 cc. to 468.03 mgm. and the insoluble sulfates from 0 to 159.39 mgm. per 100 cc. The phosphates also show an increased solubility from 5.5 per cent in 4 days, to 33.7 per cent in 8 days. But the largest amount of soluble phosphates is found in 12 days. The increase between 8 and 12 days is from 33.7 to 81.7 per cent of the total insoluble phosphates in 100 cc. of medium. This is explained by the fact that the oxidation of sulfur and production of sulfuric acid and soluble sulfates precedes the solution of the insoluble phosphates.

The transformation of the insoluble phosphates into soluble forms is a result of the production of sulfuric acid; this is accompanied by a corresponding decrease in soluble sulfates, and a rise in the amount of insoluble sulfates. The hydrogen-ion concentration and titratible acidity remain more or less stationary during the rapid transformation of the insoluble phosphates, since the acid produced is used up; but, as soon as all insoluble phosphate has been made soluble (in about 23 days), the acidity begins to increase on account of the accumulation of free acids.

The most striking thing in the experiment is the solution of the insoluble phosphate to the extent of 81.7 per cent in 12 days and 93.9 per cent in 15 days. Since these amounts were never increased, even in 68 days, we must conclude that all the insoluble phosphate became soluble in the pure culture in the period of 12 to 15 days, and the salts in the culture have come to a state of equilibrium. The fact that only 81.7 to 93.9 per cent of the phosphate is found in water solution would lead us to assume that some of the phosphate is either present in the di-calcium form and was carried down together with the crystalline calcium sulfate, or that the drying out of the culture in the prolonged experiment involved certain errors in the determinations; also some of the phosphate was assimilated by the organisms. This leads us to conclude that, in the presence of an excess of sulfur, the pure culture of the sulfur-

oxidizing organisms will convert all the insoluble phosphate, or at least 86 to 94 per cent of it, into water-soluble phosphate in a period of about 15 days, under proper experimental conditions.

After the fifteenth day, the phosphate concentration in the solution has reached an equilibrium (with due consideration to the variabilty of the individual flasks). The further oxidation of the excess of sulfur in the medium leads to the accumulation of free sulfuric acid; this acts upon the soluble calcium phosphates, transforming them into phosphoric acid and both soluble and insoluble sulfates. The physico-chemical relations of the phosphates and

TABLE 6

*Vitality of the sulfur-oxidizing organisms on continued transfer**

GENERATION	AGE OF CULTURE	pH	TITRATION
	days		*cc.*
Control		6.2	0.16
Third	7	1.8	
Third	14	1.2	
Fourth	21	1.6	
Fourth	28	1.4	
Fourth	81	0.6	3.65
Fourth	83	0.6	4.00
Fifth	7	3.6	
Fifth	14	2.0	
Fifth	68	1.9	1.70
Fifth	75	0.6	4.20
Sixth	7	4.0	
Sixth	21	2.8	
Sixth	61	1.8	2.00
Seventh	33	2.6	1.05
Seventh	46	2.6	1.10
Seventh	61	1.8	2.25
Eighth	33	2.2	1.25
Ninth	33	3.8	0.70

* Same medium (dextrose-free) was used throughout.

sulfates in solution will be taken up in detail in a later paper. For the present we will limit ourselves to general considerations. The pH of the medium gradually decreases and the titration increases, accompanied by an increase in total sulfates. In 120 days the culture is found to be equivalent to 0.68 *N* acid. Of course the acid is a mixture of sulfuric and phosphoric, and the titration indicates not only the free acid, but also the acid salts. The hydrogen-ion concentration of the solution gives a truer indication of its acidity. The pH of the solution is found in 120 days to be 0.8 (in other cases, the reaction has gone down to 0.58 pH and less), which is equivalent to more than 0.1 *N* acid all dissociated.

The organism was grown on medium I for a period of 8 months. But, on consecutive transfer, it was observed that it takes a longer period of time to bring the reaction down to the same point, as shown in table 6.

The data in the above table indicate a definite deterioration of the sulfur-oxidizing capacity of the organisms, on continued cultivation in artificial, liquid culture media. While in the third generation it took the organism 14 days to bring the acidity of the medium to a pH of 1.2, in the fifth generation it took over 68 days to produce the same acidity, and in the sixth and seventh even after 61 days, the same concentration of acid was not yet obtained. The titration of the culture, which expresses in terms of 0.1 N NaOH, how much alkali was required to neutralize 1 cc. of the culture with phenolphthalein as an indicator, runs parallel to the hydrogen-ion concentration of the culture.

To throw further light on the question of the deterioration of the culture when grown for successive generations on artificial culture media, several experiments were carried out, the results of which are reported below.

INFLUENCE OF INITIAL REACTION UPON THE GROWTH OF THE ORGANISMS

For the study of the influence of the initial reaction of the medium upon the growth of the organism and its ability to oxidize sulfur, various amounts of phosphoric acid and phosphates were added to medium I.

The series was arranged as follows:

	pH.
90 cc. of medium + 9 cc. 1.0N H_3PO_4 + 1 cc. 1.0M KH_2PO_4	2.0
90 cc. of medium + 8 cc. 1.0N H_3PO_4 + 2 cc. 1.0M KH_2PO_4	2.2
90 cc. of medium + 5 cc. 1.0N H_3PO_4 + 5 cc. 1.0M KH_2PO_4	2.5
90 cc. of medium + 3 cc. 1.0N H_3PO_4 + 7 cc. 1.0M KH_2PO_4	2.8
90 cc. of medium + 1 cc. 1.0N H_3PO_4 + 9 cc. 1.0M KH_2PO_4	3.4
90 cc. of medium + 10 cc. 1.0M KH_2PO_4	4.2
90 cc. of medium + 9 cc. 1.0M KH_2PO_4 + 1 cc. 1.0M K_2HPO_4	5.6
90 cc. of medium + 7 cc. 1.0M KH_2PO_4 + 3 cc. 1.0M K_2HPO_4	6.5

The media thus buffered, free from any insoluble phosphates or other neutralizing agents, were sterilized, inoculated alike in duplicate from the same mother culture, and incubated. At the end of 15 days the cultures were examined as to growth indicated by the turbidity formed, change in pH value, formation of soluble sulfates and the disappearance of elementary sulfur.

The reaction of the medium, properly buffered and ranging from pH=2.8 to 2.0 gave the best growth, accompanied by a greater oxidation of sulfur as indicated by the range in pH and production of soluble sulfates. A reaction corresponding to the pH value of 5.6 and more is not favorable for the development of the organism, while a reaction corresponding to a pH between these limits will allow a development of the organisms, but will probably require a greater period of time for its action.

These results have a direct practical bearing. The organism has its optimum within the limits of reaction, roughly defined between pH = 3.0 to 2.0, this, of course, depending on the concentration of buffer material in the medium. When the organism is inoculated upon the medium having an optimum reaction, it begins to develop at once and oxidize sulfur, resulting in a rapid accumulation of acid. But when the medium is at pH = 3.0 to 5.6, the organism is still able to develop, but very slowly. Particularly is the growth checked by the presence of an excess of insoluble alkaline calcium salts, because the acid produced will at first be used up in dissolving these salts, and only after the neutralizing agent has gone into solution, will the reaction be brought to a point optimum for the activities of the organism. This point is very important and we will come back to it again.

TABLE 7

Influence of initial reaction of the culture on the oxidation of sulfur

INITIAL pH	FINAL pH	SOLUBLE SULFATES AS SO_4 IN 100 CC.	TURBIDITY (GROWTH)*
		mgm.	
2.0	1.2	614.92	5
2.2	1.9	—	5
2.5	1.85	555.17	4
2.8	1.9	569.20	4
3.4	2.2	418.44	2
4.2†	{2.0 2.6}	{537.46 370.04}	3 1
5.6	5.6	265.26	T
6.5	6.5		0
Control	6.2	210.00	0

* 0 = none, T = trace, 1 = scant, 2 = poor, 3 = fair, 4 = good, 5 = very good.

† Duplicates not checking.

Since our medium no. I contains 1 gm. of tri-calcium phosphate which will tend to keep the medium less acid, until it has all been converted into mono and di-calcium salts, the question as to how this presence of calcium affects the growth and the vitality of the organism was presented. As pointed out above, the vitality of the culture continually deteriorated on constant transfer. It was then decided to isolate the organism again from the soil by using medium I, but it developed only slowly and was badly contaminated with fungi, particularly in the presence of dextrose. In view of the fact that no solid medium has been developed as yet for the isolation of the organism, the continuous growth on the liquid medium for the purpose of obtaining pure cultures was a lengthy and tedious procedure. A detailed study was then undertaken of the influence of acids, particularly sulfuric and phosphoric; bases, particularly calcium; and carbohydrates upon the growth of the sulfur-

oxidizing organism, both at the point of isolation from the composts and in continuous transfer. This resulted in the development of new culture media, which can be used for the direct isolation of the organism from the soil and for demonstrating its presence in the soil and other substrata. This will form the subject of one of the ensuing papers from this laboratory.

The organism survives in very concentrated acid solutions, equivalent to as much as 0.8 *N* acid, which is a mixture of sulfuric and phosphoric acids, and mono-calcium phosphate. The hydrogen-ion concentration of the medium may go down to as low as pH = 0.58, without killing the organism and without injuring its ability of reproduction.

The acid, however, acts destructively upon the vitality of the organism and it was found that on transferring more often (about every 4 to 7 days), when the reaction has not gone down below pH = 1.4 to 1.6, the vitality and activity will be much greater.

On the other hand, the alkalies act detrimentally upon the activities of the organism, so that the growth will be very slow in the presence of an excess of $CaCO_3$, at a reaction equivalent to pH = 6.0 and above. Growth is rather slow until enough acid has been accumulated for the complete neutralization of tri-calcium phosphate, then it proceeds at a more rapid pace. When $CaCO_3$ is used as a neutralizing agent, growth may not take place at all, because the presence of the carbonate will tend to neutralize any acid formed and keep the medium at a slightly alkaline reaction, which will prevent any development of the organism.

DESCRIPTION OF THE SULFUR-OXIDIZING ORGANISM

A detailed morphological and physiological study of the organism will be given elsewhere (10). The organism is somewhat related to the two Thiobacilli described by Beijerinck and Jacobsen and was therefore classified in the genus *Thiobacillus*. *Thiobacillus thiooxidans* is a short rod, with round edges, almost spherical, with a tendency to occur in pairs. It is less than 1 μ long and about 0.5 μ in diameter. It is autotrophic, deriving its carbon from the CO_2 of the atmosphere and its energy from the oxidation of sulfur, also thiosulfate. The presence of organic materials is not injurious to its growth; as a matter of fact, certain substances, such as dextrose, glycerin and alcohol, present in small quantities (0.1 per cent), may prove beneficial to its initial growth at least. However, this subject has not been fully investigated as yet. As nitrogen sources, it can use ammonium salts and nitrates, but since the first tend to leave the medium more acid than the second, the first are preferred. The mineral requirements are limited, so that only potassium phosphate will supply this need; however, since no care was taken as yet to eliminate the iron, magnesium and traces of other minerals from the distilled water and glass containers, it is not safe to state that these are not required.

THE IMPORTANCE OF THE ORGANISMS IN THE SOIL ECONOMY

The general relations of sulfur-oxidizing organisms in cultivated soils are not well understood. The facts already at hand would seem to indicate that these organisms are present in practically all cultivated soils. This assumption is justified by the fact that more sulfur is oxidized in unsterilized than it is in corresponding sterilized soils. Future investigations must determine how many species of sulfur-oxidizing bacteria there are and how these differ in their metabolic processes.

Many important applications are already indicated. For one thing, they can be made and have already been made an important aid in modifying soil reaction. By adjusting the quantities of sulfur employed practically any reaction within given limits may be established. Soil acidity great enough to prevent the development of the potato scab fungus can be readily created. Yet, the acidity thus developed need not be intense enough to interfere with the production of large crops of clean potatoes. By the use of still larger quantities of sulfur the soil may be made so acid as to prevent the development of soil-infesting insects, including such troublesome insects as wireworms and white grubs. The degree of soil acidity may be raised to a point where undesirable weeds or other plants would be eliminated. It would be possible later to correct this acidity by the use of lime or of other basic materials.

There is promise that, through the use of sulfur and suitable cultures of sulfur-oxidizing bacteria, black alkali soils may be reclaimed. In this case the injurious carbonate and bicarbonate of sodium would be gradually changed into the less injurious sodium sulfate. Preliminary experiments already carried out in our laboratories show that this is quite feasible.

The sulfuric acid produced when sulfur is oxidized in the soil may serve as an effective solvent. The supply of available phosphates and of potassium and calcium or magnesian compounds may thus be readily increased. In certain soil regions the use of sulfur and of sulfur-oxidizing bacteria for this purpose may be of considerable moment.

There are extensive areas of soil that respond readily to applications of sulfates or of sulfur. The investigations at the Oregon Agricultural Experiment Station show that elementary sulfur may be used to great advantage in increasing the yields of alfalfa and of clover. Crop increases through the use of sulfur also have been obtained in other localities, although the results have not been so consistent. There is considerable evidence at hand to the effect that inoculated sulfur may be used to very considerable advantage in the growing of garden crops, even where atmospheric precipitation may supply considerable quantities of sulfur.

Composting experiments have shown that soluble phosphates may be readily produced when mixtures of mineral phosphates, of sulfur and of sulfur-oxidizing bacteria are kept under suitable conditions as to moisture, tempera-

ture and aeration. In the same way, soluble potash compounds have been produced from greensand marls containing the mineral glauconite. A number of these points are under investigation at the New Jersey Agricultural Experiment Station and the data as accumulated will be reported on in future papers.

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PRACTICAL SIGNIFICANCE OF THE ORGANIC CARBON NITROGEN RATIO IN SOILS

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In connection with certain investigations on the quantitative relationships of soil organic matter it appeared worth while to consider the significance of the organic carbon-nitrogen ratio in its relation to soil productivity. The results for carbon were obtained by the rapid dry combustion method as modified by Read (7) for the simultaneous determination of soil organic matter and organic carbon. The carbon determinations have already been presented in the preceding paper on the conventional carbon factor. The nitrogen determinations were made by the official Kjeldahl method.

Some recent studies have been reported on the carbon-nitrogen ratio as it occurs in various soils, and several investigators have suggested that certain given ratios represent certain degrees of fertility.

LITERATURE CONSULTED

Pagnoul (6) found no fixed relation between carbon and nitrogen, but the carbon, nitrogen and humus content all appeared to vary in the same direction, although irregularly.

Dyer (3) studied the carbon-nitrogen ratio of 21 Rothamstead wheat soils at 10 successive depths of 9 inches each. For the first, second, third, fourth, and fifth depths the corresponding carbon-nitrogen ratios for all plats varied as follows: 9 to 10; 7.6 to 9.7; 6.7 to 8.2; 5.6 to 7.8; 5.0 to 7.1. At the fifth and sixth depths the ratio ceased to become narrower and was little influenced by past surface accumulations.

Kaserer (5) showed that for every soil there was a certain state of equilibrium between the nitrogen and the carbon contained in the humus, which was influenced by the chemical composition and reaction of the soil and the conditions of moisture and temperature.

Stewart (8) stated that in general the carbon-nitrogen ratio of the soil tended to become narrower with the aging of the organic material, but that it never became narrower or even equal to the ratio of the proteins commonly present in humus-forming materials. He found the carbon-nitrogen ratios of the ordinary brown silt loam soils of Illinois to be 12.1, 11.5 and 8.9 in the surface, subsurface and subsoil, respectively, and in the black clay loam soils the corresponding ratios were 11.7, 11.9, and 9.

Alway and Vail (1) studied the relative amounts of nitrogen, carbon and humus in some Nebraska soils and found that the carbon-nitrogen ratio decreased through the first 3 feet in the upland prairie soils, but remained constant in the lowland fields until the black soil had been passed through. In the surface 6 or 12 inches of the prairie soils the carbon-nitrogen ratio varied from 10.2 to 12.7. The ratio of 12-inch depths in the cultivated fields was considerably lower than in the adjacent prairies. They concluded that the ratios for the surface soils were sufficiently constant to estimate the carbon content from a nitrogen determination, thereby making a carbon determination unnecessary for practical purposes, and that the percentage of humus in a soil could be estimated from its nitrogen content if similar soils from that part of the state had been analysed.

Felber (4) studied the equilibrium between the nitrogen and carbon in 13 soils, all of different geological formation and composition. His findings corroborated those of Kaserer. In the surface of arable soils the ratio varied from 8.3 to 10. The ratio was 10 in soils very rich in humus and varied from 6.6 to 7.7 in soils poor in humus. In the subsoils the proportions varied between 11 and 14 and the amounts of carbon and nitrogen decreased with the depth. His results indicated that the carbon-nitrogen ratio was sufficiently constant for different soils to encourage the elaboration of a method to determine approximately the amount of humus in a soil from the percentage of nitrogen found in that soil. Such a method implied the use of the conventional carbon factor.

Brown and Allison (2, p. 49) stated:

> The determination of the carbon-nitrogen ratio in soils is now coming to be considered of much importance in fertility studies. Not only does it show the organic matter content of soils more accurately than the more or less arbitrary humus determinations concerning which considerable difference of opinion exists, but it also throws some light upon the rate at which decomposition processes are occurring in the soil.
>
> Experience has shown that (in the case of humid soils) if the ratio narrowed beyond a point of about 1 to 10 crop yields may be reduced, evidently because of an insufficient production of available nitrogen, phosphorus and potassium. On the other hand, if the ratio was 1 to 12 or above, bacterial activities apparently occur to a satisfactory extent and sufficient amounts of soluble plant-food are produced for good crop growth.

Waynick and Sharp (9) presented data showing the amount of variation found in the total carbon and the total nitrogen in the silty clay loam soil at Davis and in the blow sand at Oakley, California. A plat of about 1.3 acres was selected at each place, the choice being made on apparent uniformity, and 100 soil samples were taken from each plot. In the Davis soils the nitrogen varied from 0.977 to 0.124 per cent and the carbon ranged from 0.896 to 1.383, while in the Oakley soil the percentage range for nitrogen was from 0.021 to 0.063 and for carbon from 0.179 to 0.947. These variations indicate the unreliability of results secured from one or possibly several determinations. The carbon-nitrogen ratios calculated from the analytical

TABLE 1

Relation of carbon-nitrogen ratio to yields of corn and wheat on 37 surface soils

REGISTER NUMBER	SOIL AND LOCATION	DEPTH SAMPLE	ORGANIC MATTER	ORGANIC CARBON	ORGANIC NITROGEN	C:N RATIO	AVERAGE ANNUAL YIELD PER ACRE	
							Corn	Wheat
		inches	*per cent*	*per cent*	*per cent*		*bu.*	*bu.*
2654	Lamoure silty clay loam (Iowa)	0–20	6.18	54.78	0.323	10.48	44.	20
2656	Webster silty clay loam (Iowa)	0–15	3.62	50.62	0.203	9.03	40	20
2658	Wabash silt loam (Iowa)	0–20	6.18	55.44	0.362	9.46	42	
2660	Carrington loam (Iowa)	0– 8	4.20	54.26	0.284	8.03	33	17
2662	Fargo silty clay loam (Iowa)	0–18	6.82	53.53	0.359	10.17	31	17
2669	Shelby loam (Mo.)	0–10	1.71	50.67	0.130	6.66	35	
2672	Decatur silt loam (Mo.)	0–10	1.68	47.07	0.129	6.13	31	
2675	Grundy silt loam (Mo.)	0–12	2.70	52.93	0.207	6.90	40	
2678	Marshall silt loam (Mo.)	0–12	2.37	50.07	0.162	7.32	45	
2682	Summit silt loam (Kan.)	0– 7	4.07	53.54	0.261	8.35	27	16
2684	Laurel very fine sandy loam (Kan.)	0– 7	2.73	54.41	0.165	9.00	30	18
2686	Marshall silt loam (Kan.)	0– 7	2.68	53.42	0.185	7.74	24	17
2688	Wabash silty clay loam (Kan.)	0– 7	4.22	53.54	0.218	10.32	35	20
2690	Oswego silt loam (Kan.)	0– 7	5.59	53.32	0.282	10.57	23	16
2712	Fargo clay loam (Minn.)	0– 8	6.12	55.09	0.401	8.41	31	21
2714	Gloucester loam (Minn.)	0– 8	1.72	50.66	0.113	7.71	50	
2716	Carrington silt loam (Minn.)	0– 8	4.22	49.92	0.248	8.49	48	21
2718	Hempstead silt loam (Minn.)	0– 8	3.76	54.43	0.211	9.69	50	25
2720	Colby silt loam (Wis.)	0– 7	1.91	48.77	0.119	7.83	40	
2722	Clyde sandy loam (Wis.)	0– 7	3.70	54.54	0.228	8.85	35	
2724	Clyde loam (Wis.)	0– 7	5.26	54.64	0.361	7.96	44	
2726	Clyde silt loam (Wis.)	0– 7	4.56	52.67	0.278	8.64	53	
2728	Superior silt loam (Wis.)	0– 7	2.00	44.61	0.141	6.32	44	
2730	Waukesha silt loam (Wis.)	0– 7	1.23	44.38	0.121	4.51	46	
2732	Knox silt loam (Wis.)	0– 7	2.18	45.21	0.156	6.32	42	
2734	Westmoreland silty clay (W. Va.)	0– 7	5.40	50.49	0.308	8.85	50	
2738	Upshur clay (W. Va.)	0– 7	2.73	30.20	0.129	6.16	30	
2740	Moshannon silt loam (W. Va.)	0– 7	2.44	33.01	0.153	5.26	40	
2742	Huntington loam (W. Va.)	0– 7	5.34	36.89	0.282	6.98	45	
2744	DeKalb silt loam (W. Va.)	0– 7	2.47	37.56	0.146	6.35	25	
2746	Tyler silt loam (W. Va.)	0– 7	2.44	40.34	0.156	6.30	40	
2748	Wheeling silt loam (W. Va.)	0– 6	3.50	34.51	0.191	6.32	40	
2760	Barnes loam (N. Dak.)	0– 7	6.18	55.87	0.368	9.38	35	
2764	Fargo clay (N. Dak.)	0– 7	8.05	53.51	0.401	10.74	45	
2766	Bearden silt loam (N. Dak.)	0– 7	5.46	55.08	0.356	8.45	40	
2791	Diablo clay (Cal.)	0–12	3.30	56.27	0.183	10.15		18
2792	Diablo clay adoba (Cal.)	0–24	1.38	49.02	0.083	8.15		10

data reported for these extremes show a variation of 10.9 to 12.5 in the Davis soil and 8.1 to 20.4 in the Oakley soil. Such variation as these occurring on small areas of soil selected for their apparent uniformity would seem to discredit to a considerable extent any significance that might be given to the correlation of the carbon-nitrogen ratio with soil fertility, especially if only a relatively few samples are considered.

EXPERIMENTAL

The data presented in table 1 show the carbon-nitrogen ratio for 37 different soils, the average annual production of corn on all of these soils except the two from California, and the yield of both corn and wheat on twelve of the soils. The values for the carbon-nitrogen ratios range from 10.74 (Fargo clay) to 4.51 (Waukesha silt loam). In general the lower ratios are associated with the lower percentages of organic matter, but no correlation is shown between crop yields and the ratio of carbon to nitrogen. In fact the lowest yield of corn, 23 bushels per acre, is on soil 2690, Oswego silt loam, which has a ratio of 10.57, and the Waukesha silt loam, no. 2730, with the lowest ratio in the table, 4.51, produces 46 bushels of corn per acre. Although these are extreme variations it is very evident from an inspection of the table that a given ratio does not indicate a certain degree of productivity.

The data for the crop yields given above represent as accurate information as could be obtained from the soils divisions of the stations which furnished samples. None of the yields reported have been influenced by the use of commercial fertilizers, and practically all the samples of the different soils studied were taken in the regular soil survey work of the different stations and should be representative of the various soil types.

SUMMARY

Sixteen of the 37 soils studied contain less than 3 per cent of organic matter, and the carbon-nitrogen ratio for these soils ranges from 4.51 to 9.00. The yield in bushels of corn per acre varies from 24 to 50 on soils whose ratios are 7.74 and 7.71 respectively.

The remaining 21 soils, having above 3 per cent of organic matter, show a variation of from 6.32 to 10.74 in the ratio of carbon to nitrogen, and the yield of corn given for each of these extremes is 40 and 45 bushels, respectively. The ratio for the lowest yield of corn on the 21 soils, 23 bushels per acre, is 10.57, while that for the highest yielding soil, 53 bushels per acre, is 8.64.

A consideration of the data presented in this paper leads to the conclusion that soil productivity cannot be correlated with the organic carbon-nitrogen ratio.

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